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# THE GERMICIDAL PROPERTIES OF ALKALINE WASHING SOLUTIONS, WITH SPECIAL REFERENCE TO THE INFLUENCE OF HYDROXYL-ION CONCENTRATION, BUFFER INDEX, AND OSMOTIC PRESSURE [1]

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## INTRODUCTION

The use of the automatic bottle washer for cleaning milk bottles has become an almost universal practice in city milk plants. The bottle-washing machine combines what was formerly two processes—cleaning and sterilizing. Most of the solutions used in these machines depend for their germicidal action either upon a high concentration of alkali or upon a combination of an alkaline washing solution and a sterilizing rinse, such as a hypochlorite.

The present investigation was undertaken to determine some of the factors that influence the germicidal efficiency of various alkalies and basic salts. That the disinfectant action of an alkali is due largely to the concentration of free hydroxyl ions in solution has been generally accepted, though many investigators have pointed out that other factors must be considered also. Important among these factors are other ions and molecules (*20*, *18*, *25*)[2] and the physical forces of surface tension, adsorption electrical potential swelling, and osmotic effects (*26*).

Paul and Krönig (*21*) in their classic experiments on disinfection concluded that the disinfecting efficiency of the alkali hydrates is in proportion to their degree of electrolytic dissociation and that the concentration of hydroxyl ions is responsible for the germicidal action. Potassium, sodium, and lithium hydroxides, they found, were powerful disinfectants, but sodium hydroxide was slightly less effective than potassium hydroxide and lithium slightly less than sodium. On the other hand, the weakly dissociated ammonium hydroxide was of almost no value.

The relation of the degree of electrolytic dissociation of certain toxic substances to their destructive action has been studied by several investigators. Dreser (*8*), Paul and Krönig (*21*), Chick (*7*), and Madsen and Nyman (*15*) have shown that in the case of mercury

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[2] Reference is made by number (italic) to "Literature cited," p. 562.

salts the disinfecting action was proportional to the mercury-ion concentration and was decreased by the addition of sodium chloride, due to depression of the ionization of the mercury salts.

That acids owe their germicidal action, at least partly, to their degree of electrolytic dissociation has been shown by Paul and Krönig (*21*), Bial (*4*), and Winslow and Lockridge (*29*). Paul, Birstein, and Reuss (*19*) advanced the view that not only the hydrogen-ion concentration but also the anions and undissociated molecules of the acids play an important part in the disinfection process. They considered that the anions and undissociated molecules accelerated the action of the hydrogen ions. Paul, Birstein, and Reuss (*20*) further found that the disinfectant action of inorganic acids was accelerated by inorganic salts in a concentration in which they exerted no disinfectant action. This was found to hold when the salt contained a common anion as well as when it contained a different anion from that of the acid. Norton and Hsu (*18*) concluded that the addition of a salt containing a common anion, because it decreased the hydrogen-ion concentration and increased the concentration of undissociated acid molecules, diminished the disinfectant action. Salts which had no appreciable effect on the dissociation of an acid increased the disinfecting properties of the acid. They suggest on the basis of their finding (and in this they differ from Paul, Birstein, and Reuss) that acid anions are positive catalyzers and undissociated acid molecules are negative catalyzers in acid disinfection.

Weiss (*27*) showed that an increase in hydroxyl-ion concentration lowered the thermal resistance of *Clostridium botulinum*. He pointed out that the destruction of the spore is a gradual process and not an instantaneous killing and is probably due to a protein coagulation. Just as the thermal death rate of *C. botulinum* increased with increasing hydroxyl-ion concentration, the thermal death rate also increased with increasing concentration of hydrogen ions. Sodium chloride, it was found, also increased the thermal death rate.

An increase in pH on the alkaline side of neutrality, as was shown by Myers (*17*), increased the power of a solution to destroy *Bacterium coli* at a given temperature. It was also observed that different buffer mixtures of approximately the same pH value exert very different germicidal effects.

Mudge and Lawler (*16*) have found that with the same chemical compound germicidal action is well correlated with the pH of the solution. They have further shown the effect of concentration on germicidal action. Solutions of sodium carbonate were less efficient than solutions of sodium hydroxide of the same concentration. But the germicidal action of the less alkaline solutions was greatly increased by increasing the concentration of the solute, even though the pH remained relatively low. This agrees with the results obtained by Levine, Peterson, and Buchanan (*11*, *12*).

Levine, Toulouse, and Buchanan (*13*) have also found that the addition of sodium chloride, sodium carbonate, or trisodium phosphate to sodium hydroxide increases the germicidal action of sodium hydroxide. When equal weights were used, they found that sodium chloride and sodium carbonate gave the same effect, while trisodium phosphate was less efficient. One suggestion that these investigators make in explanation is that the addition of the various salts would tend to decrease the dissociation of sodium hydroxide and would

thus increase the concentration of undissociated sodium hydroxide. This is contradicted by their previous statement that "there was no significant change in the H-ion concentration due to the addition of these salts" (sodium chloride and sodium carbonate in concentrations of 1, 2, and 3 per cent). Nevertheless, they explain the action by the suggestion that the undissociated sodium hydroxide rather than the hydroxyl ion is the agent which penetrates the bacterial cell. One other suggestion they make is that the salts decrease the solubility of sodium hydroxide in the water phase and that the sodium hydroxide is thus forced into the bacterial phase of the suspension.

Among the many interesting questions that were suggested by previous investigations, the following appeared to be of especial importance: (1) How much do solutions of different commercial washing powders vary in germicidal action? (2) To what extent are alkali washing solutions dependent upon the hydroxyl-ion concentration for their germicidal action? (3) What are some of the other important factors that influence germicidal action of alkali solutions and how may they be measured? It was with these questions in mind that the experimental work was started.

## PRELIMINARY EXPERIMENTS ON GERMICIDAL ACTION OF ALKALINE WASHING SOLUTIONS

### MATERIALS AND METHODS

Cultures of *Bacterium coli* grown in 1 per cent peptone for three days at 25° C. were used as the test cultures, except where the age of the culture is otherwise indicated. For at least five days previous to the test, the cultures had been transferred daily in 1 per cent peptone solutions. The strain of *Bact. coli* that was used came from the Iowa State College (culture No. 24), and was carried on agar slants previous to the investigation. This organism gave the typical *coli* reactions.

The culture of *Bacillus cereus* used was one isolated from soil at the New York State College of Agriculture. Cultures were grown on standard nutrient-agar slants for seven days at 37° C. Just before the test was made, the growth was washed off the slant with 10 c. c. of sterile 0.85 per cent sodium chloride solution. This suspension of bacteria was then heated at 80° for 10 minutes to kill the vegetative form of the organism. The spore suspension was cooled and held at 55° while the disinfection test was being made. The count obtained when this suspension was plated out was taken as the number of spores per cubic centimeter.

All washing solutions which were tested were made up and sterilized not more than 12 hours before each test. The pH of the solution was determined with a hydrogen electrode immediately after each experiment except in the case of powder D, which contained a strong oxidizing agent that prevented the determination of the pH electrometrically. A rough determination was made colorimetrically. A type K Leeds & Northrup potentiometer was used for the electrometric determinations, and the hydrogen electrode was checked frequently.

EXPERIMENTS WITH FOUR WASHING-POWDER SOLUTIONS OF DIFFERENT pH VALUES

Freshly prepared and sterilized 1 per cent solutions were used in each test. A 3-day-old culture of *Bacterium coli* in 1 per cent peptone was plated out on a standard agar before the test was made. A measured volume of the culture was then introduced with a pipette into the solution to be tested, so that the culture was diluted ten times. At various intervals samples of the suspension were drawn with a pipette and plated out on standard agar. All plates were incubated for 48 hours at 37° C. By this means the rates at which the bacteria were killed in the different solutions were determined. In Tables 1 to 4 are given the results obtained, for 25° and for 45° as the temperatures of exposure. It is readily seen from the tables that there is considerable difference in the germicidal properties of these solutions.

TABLE 1.—*Effect of pH and temperature of solution of washing powder E in 1 per cent concentration on rate of disinfection when 3-day-old culture of Bacterium coli incubated at 25° C. was used*

| Rate of disinfection at a— | | | | | |
|---|---|---|---|---|---|
| pH of 10.04 and a temperature of 25° C. | | pH of 11.94 and a temperature of 25° C. with sodium hydroxide added | | pH of 10.07 and a temperature of 45° C. | |
| Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter |
| 0 | 28, 400, 000 | 0 | 34, 000, 000 | 0 | 36, 000, 000 |
| 1 | 21, 600, 000 | ½ | 0 | 1 | 20, 000, 000 |
| 2 | 24, 500, 000 | 1 | 0 | 2 | 10, 200, 000 |
| 3 | 26, 700, 000 | 5 | 0 | 5 | 0 |
| 5 | 25, 800, 000 | | | 10 | 0 |
| 10 | 21, 000, 000 | | | 20 | 0 |
| 20 | 15, 000, 000 | | | | |

TABLE 2.—*Effect of pH and temperature of solution of washing powder C in 1 per cent concentration on rate of disinfection when 3-day-old culture of Bacterium coli incubated at 25° C. was used*

| Rate of disinfection at a— | | | | | |
|---|---|---|---|---|---|
| pH of 10.70 and a temperature of 25° C. | | pH of 11.74 and a temperature of 25° C. with sodium hydroxide added | | pH of 10.80 and a temperature of 45° C. | |
| Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter |
| 0 | 32, 100, 000 | 0 | 35, 200, 000 | 0 | 34, 800, 000 |
| 1 | 16, 800, 000 | ½ | 100 | ½ | 200 |
| 2 | 15, 900, 000 | 1 | 150 | 1 | 0 |
| 3 | 6, 100, 000 | 5 | 0 | 2 | 0 |
| 5 | 3, 000, 000 | | | 5 | 0 |
| 10 | 270, 000 | | | | |
| 20 | 1, 000 | | | | |

TABLE 3.—*Effect of pH and temperature of solution of washing powder G in 1 per cent concentration on rate of disinfection when a 3-day-old culture of Bacterium coli incubated at 25° C. was used*

| Rate of disinfection at a— | | | | | |
|---|---|---|---|---|---|
| pH of 10.59 and a temperature of 25° C. | | pH of 11.98 and a temperature of 25° C. with sodium hydroxide added | | pH of 10.64 and a temperature of 45° C. | |
| Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter |
| 0 | 29, 200, 000 | 0 | 39, 300, 000 | 0 | 33, 100, 000 |
| 1 | 20, 700, 000 | ½ | 0 | ½ | 122, 000 |
| 2 | 8, 400, 000 | 1 | 0 | 1 | 50 |
| 3 | 3, 490, 000 | 5 | 0 | 2 | 0 |
| 5 | 540, 000 | | | 5 | 0 |
| 10 | 900 | | | | |

TABLE 4.—*Effect of temperature of solution of washing powder F in 1 per cent concentration on rate of disinfection when a 3-day-old culture of Bacterium coli incubated at 25° C. was used*

| Rate of disinfection at a— | | | |
|---|---|---|---|
| pH of 11.70 and a temperature of 25° C. | | pH of 11.64 and a temperature of 45° C. | |
| Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter |
| 0 | 37, 100, 000 | 0 | 37, 300, 000 |
| ½ | 250 | ½ | 500 |
| 1 | 0 | 1 | 0 |

In order to see whether the pH was responsible for the germicidal power of the solution, sodium hydroxide was added to powders E, C, and G till the pH of each of these solutions was near that of powder F. The three solutions were then tested as above; the results are given in Tables 1, 2, and 3. About the same length of time was required for each powder to destroy the bacteria when the pH values of the solutions were approximately the same. In Table 1 it is seen that the germicidal action of powder E in 1 per cent concentration is very slight at 25° C., and that its destructive action is increased by an increase in temperature to 45°. Increasing the pH from 10.04 to 11.94 renders this solution extremely effective, even at 25°.

Table 2 shows that powder C fails to destroy the suspension of *Bacterium coli* at 25° C., even after 20 minutes, while at 45° complete destruction is brought about within 1 minute. When the pH is increased from 10.70 to 11.71, the germicidal action is greatly increased, and within 1 minute nearly complete destruction results.

In Table 3 are recorded the results with powder G. These results are similar to those obtained on powders E and C. Raising the pH of the solutions of powders E, C, and G by the addition of sodium hydroxide so that the pH values are approximately the same as the

pH of the solution of powder F, increases the germicidal properties of the first three powders to the point where all four powders have nearly the same killing action on *Bacterium coli*.

The accelerating effect of the increase in temperature is also clearly shown. In Table 4 the apparent failure of temperature increase to accelerate the killing action is due to experimental error. As the time necessary for complete disinfection becomes shorter, the difficulty of accurate measurement increases.

### EXPERIMENTS TO DETERMINE THE EFFECT OF DILUTION ON THE GERMICIDAL PROPERTIES OF WASHING SOLUTIONS

A culture of *Bacterium coli*, grown three days at room temperature, was used for the test. The solution to be tested was inoculated with a measured volume of the culture which was plated out on standard agar at the beginning of the experiment. Tenth of a cubic centimeter samples of the bacterial suspension to be tested were transferred to tubes of 1 per cent peptone at different intervals, that is, at ½, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, and 20 minutes. The tests were run in duplicate. Growth was determined by the turbidity of the peptone medium after 48 hours' incubation at 37° C. Tests were made to determine whether the peptone solution was made unfavorable for growth by the addition of the test solution. In each case the medium proved favorable for growth. The results are recorded in Table 5. Solutions of the powders were made up in dilutions of 1:100, 1:500, 1:1,000, and 1:10,000. These solutions were sterilized in the autoclave as usual.

TABLE 5.—*Effect of dilution on the germicidal properties of washing solutions when a 3-day-old culture of Bacterium coli incubated at 25° C. was used*

[Count of suspension at start, 4,000,000 per cubic centimeter]

| Washing powder | Temperature (° C.) | Dilution | pH | Time necessary for complete destruction of bacteria (minutes) |
|---|---|---|---|---|
| E | 45 | 1:100 | 10.03 | 3. |
| | | 1:1,000 | 9.89 | 15. |
| | | 1:10,000 | 8.27 | Not destroyed in 20. |
| G | 45 | 1:100 | 10.80 | 1. |
| | | 1:1,000 | 10.08 | 10. |
| | | 1:10,000 | 8.28 | Not destroyed in 20. |
| D | 25 | 1:100 | * 11.5 | ½ |
| | | 1:500 | * 10.0 | 1. |
| F | 25 | 1:100 | 11.78 | 1. |
| | | 1:500 | 11.01 | Not destroyed in 15. |
| C | 25 | 1:100 | 11.00 | 6. |
| | | 1:1,000 | 10.50 | Not destroyed in 30. |
| A | 25 | 1:100 | 12.73 | ½ |
| | | 1:1,000 | 11.75 | 20. |

* Approximate.

In every case it is seen that dilution decreases the germicidal efficiency, as would be expected, but not to the same extent in all the powders. For instance, powder D, in which chlorine is the important agent and in which the hydroxyl-ion concentration is a less important factor, when diluted 5 times, is still very effective in the destruction of *Bacterium coli*. On the other hand, powder F, when diluted only five times, loses very markedly its germicidal properties, even though

the pH remains as high as 11.0. Likewise, powder A, after being diluted ten times, has a high pH, that is, 11.75, and yet exerts only a weak germicidal action. Powder F, in a dilution of 1 : 100, has a pH of 11.78, almost identical with the pH of powder A in a dilution of 1 : 1,000. The former, however, is many times more effective than the latter.

From previous results we should expect that if a high pH were maintained the solution would continue to be effective as a germicide. We might expect from the observations on the effect of the hydroxyl-ion concentration on the death rate of bacteria that if we used enough of a highly alkaline powder, such as powder A, to maintain a pH equal to that of a more concentrated solution of a less alkaline powder, such as powder F, we should have approximately the same germicidal efficiency. That this is not the case, however, must be concluded from Table 5. Certainly there are other factors entering which have not been considered. Concentration of salts, the presence of toxic substances other than the hydroxyl ion, surface tension, buffer value, or possibly osmotic pressure, may be responsible for these differences.

### EXPERIMENTS WITH BACILLUS CEREUS

In order to test the effect of various washing solutions on spore-forming bacteria, the following experiment was performed. The procedure given above for *Bacterium coli* was followed in exposing the spores of *Bacillus cereus* to the different solutions. One-tenth of 1 c. c. samples of the test mixture were transferred at various intervals into 1 per cent peptone and incubated at 37° C. Powders B, D, and E, the solutions of which gave pH values of 12.68, 11.6 (approximate), and 9.98, respectively, were tested. The results of these tests showed that an exposure of 30 minutes at 25° was not sufficient for even so alkaline a solution as that of powder B to destroy spores of *B. cereus*.

In Table 6 are recorded the results of experiments made in connection with some other work, which show the effect of temperature in increasing the germicidal effect of an alkaline solution.

TABLE 6.—*The death rate of spores of Bacillus cereus at 80° C. in Ringer's $Na_2HPO_4$–NaOH mixture diluted 1:1 and adjusted to three pH values*

| Death rate of spores at— | | | | | |
|---|---|---|---|---|---|
| pH 10.35 | | pH 11.53 | | pH 12.05 | |
| Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter | Time of exposure in minutes | Number of survivors per cubic centimeter |
| 0 | 1,750 | 0 | 20,000 | 0 | 10,200 |
| 2 | * 1,800 | 1 | 10,800 | 1 | 2,500 |
| 4 | * 2,400 | 5 | 4,600 | 5 | 150 |
| 8 | 1,500 | 10 | 2,400 | 10 | 0 |
| 12 | 1,300 | 20 | 300 | 20 | 0 |
| 16 | 1,100 | | | | |
| 20 | 750 | | | | |

* The apparent increase in numbers is probably due to the breaking up of clumps and chains by shaking and not to actual increase in numbers.

Ringer's $Na_2HPO_4$–NaOH mixture, diluted 1:1, was used as the medium of exposure. After the suspension of spores of *Bacillus cereus* was prepared, as described above, 10 c. c. of the exposure medium were inoculated with 1.0 c. c. of the suspension of spores. At various intervals samples of the mixture in the exposure tube were plated out on standard agar. Counts were made after 24 hours' incubation at 37° C.

Table 6 shows the results obtained at 80° C. at different pH values. This temperature in a nearly neutral solution does not kill spores even when the time of exposure is much longer than in this experiment. Likewise solutions of a pH higher than that of any of the solutions used in these three experiments did not destroy *Bacillus cereus* at a low temperature. But when the two are combined, conditions become decidedly destructive.

## INFLUENCE OF HYDROXYL-ION CONCENTRATION, BUFFER INDEX, AND OSMOTIC PRESSURE ON GERMICIDAL ACTION OF ALKALINE SOLUTIONS

### METHODS AND MATERIALS

After the preliminary experiments described above had been made, the following study was undertaken to clear up some of the points that could not be satisfactorily explained. The study of the germicidal properties of alkaline solutions resolved itself into a more detailed consideration of at least three factors, namely, hydroxyl-ion concentration, buffer value, and osmotic pressure of the solution. The chemical compounds selected as representative of the materials most commonly used in washing powders were sodium hydroxide, sodium carbonate, and trisodium phosphate. It had been found in a few preliminary tests that the surface tension of these alkali solutions varied so slightly that this factor did not need to be considered.

The first step in this study was to determine the titration curves (figs. 1 and 2) of solutions of sodium hydroxide, sodium carbonate, and trisodium phosphate, and mixtures (in equal normalities) of sodium hydroxide and sodium carbonate and of sodium hydroxide and trisodium phosphate. These curves made possible the selection of a number of solutions in which pH, buffer value, and osmotic pressure varied considerably.

The plan followed in selecting the solutions to be tested for germicidal properties was as follows. Using the curves in Figures 1 and 2, mixtures were selected which gave certain pH values. For instance, the most alkaline solution was that of 0.5 normal sodium hydroxide, which gave a pH near 13.4. At about pH 13.1, 4 mixtures were selected from the curves. At about pH 12.2 and at about pH 11.3, other mixtures were selected. These solutions, as will be shown later, presented a considerable range of variation in pH, buffer index, and osmotic pressure. A few other solutions were also selected, making in all 18 solutions of different composition. The pH, buffer index, and osomtic pressure were determined for each of the solutions selected.

### TITRATIONS

Solutions of the compounds and mixtures were prepared so that they were equivalent to normal hydrochloric acid when methyl orange was used to determine the end-point of the titration. Twenty-

five cubic centimeters amounts of these normal alkali solutions were measured out and increasing amounts of normal hydrochloric acid were added and the volume made up to 50 c. c. Obviously,

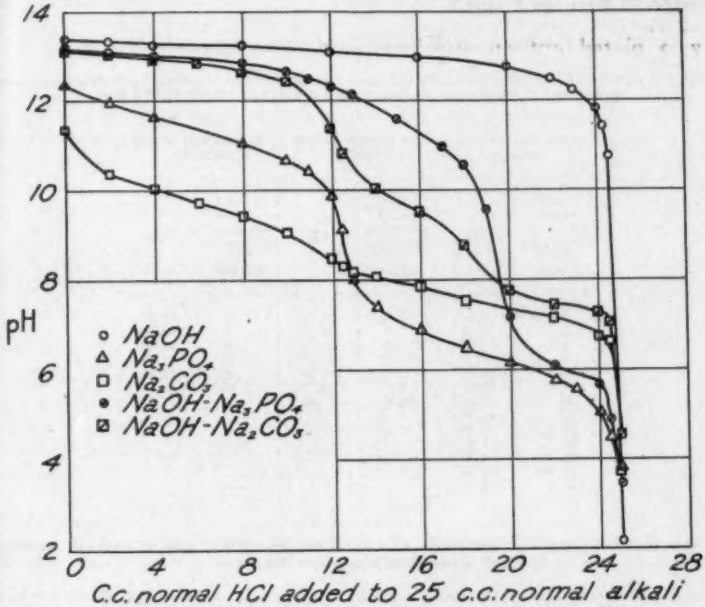


FIGURE 1.—Titration curves of solutions of sodium hydroxide, sodium carbonate, trisodium phosphate and mixtures thereof when titrated with normal HCl

then, the final solutions were 0.5 normal with respect to the alkali originally present. "Normal trisodium phosphate" has been recorded as one of the solutions. This is more than a saturated solution at 25° C. It was handled as follows: The solution was warmed to dissolve all the salt, and was then cooled to near room temperature, and the desired volumes measured out before precipitation took place. Later a 0.75 normal $Na_3PO_4$ solution was used instead of normal. The pH determinations were made at 25° C., using a hydrogen electrode of the type described by Bailey (*1*) and a type K Leeds & Northrup potentiometer equipped with a

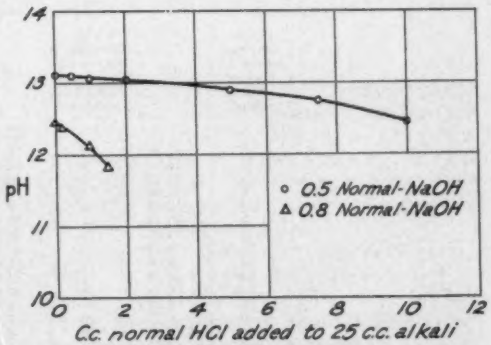


FIGURE 2.—Titration curves of sodium hydroxide solutions when titrated with normal HCl

type R galvanometer. E. M. F. readings were converted to pH by the use of the table of Schmidt and Hoagland (*24*). The results of these determinations are recorded in Tables 7, 8, and 9 and are plotted in Figures 1 and 2.

TABLE 7.—*Electrometric titrations of sodium hydroxide solutions*

[To 25 c. c. portions of solution of normality indicated various amounts of normal hydrochloric acid were added, and mixtures were made up to 50 c. c. volumes with distilled water]

| 1.0 normal NaOH solution | | 0.5 normal NaOH solution | | 0.08 normal NaOH solution | |
|---|---|---|---|---|---|
| Cubic centimeters of 1.0 normal HCl added | pH | Cubic centimeters of 1.0 normal HCl added | pH | Cubic centimeters of 1.0 normal HCl added | pH |
| 0 | 13.36 | 0.0 | 13.12 | 0.0 | 12.45 |
| 2 | 13.35 | .5 | 13.10 | .05 | 12.44 |
| 4 | 13.26 | 1.0 | 13.06 | .10 | 12.40 |
| 8 | 13.22 | 2.0 | 13.05 | .15 | 12.42 |
| 12 | 13.06 | 5.0 | 12.89 | .20 | 12.39 |
| 16 | 12.97 | 7.5 | 12.73 | 1.00 | 12.11 |
| 20 | 12.71 | 10.0 | 12.43 | 1.50 | 11.83 |
| 22 | 12.49 | | | | |
| 23 | 12.20 | | | | |
| 24 | 11.80 | | | | |
| 24.25 | 11.40 | | | | |
| 24.50 | 10.74 | | | | |
| 25 | 2.18 | | | | |

TABLE 8.—*Electrometric titrations of trisodium phosphate and of sodium hydroxide–trisodium phosphate solutions*

[To 25 c. c. portions of solution of normality indicated various amounts of normal hydrochloric acid were added and mixtures were made up to 50 c. c. volumes with distilled water]

| 1.0 normal $Na_3PO_4$ solution | | 1.0 normal solution containing NaOH (0.5 normal) and $Na_3PO_4$ (0.5 normal) | |
|---|---|---|---|
| Cubic centimeters of 1.0 normal HCl added | pH | Cubic centimeters of 1.0 normal HCl added | pH |
| 0 | 12.36 | 0 | 13.17 |
| 2 | 11.98 | 2 | 13.08 |
| 4 | 11.64 | 4 | 12.97 |
| 8 | 11.03 | 8 | 12.81 |
| 10 | 10.66 | 10 | 12.64 |
| 11 | 10.41 | 11 | 12.48 |
| 12 | 9.87 | 12 | 12.30 |
| 12.5 | 9.12 | 13 | 12.13 |
| 13 | 8.00 | 15 | 11.57 |
| 14 | 7.39 | 17 | 10.97 |
| 16 | 6.89 | 18 | 10.56 |
| 18 | 6.47 | 19 | 9.55 |
| 20 | 6.17 | 20 | 7.15 |
| 22 | 5.75 | 22 | 6.08 |
| 23 | 5.53 | 24 | 5.63 |
| 24 | 5.04 | 24.5 | 4.87 |
| 24.5 | 4.49 | 25 | 3.47 |
| 25 | 3.80 | | |

TABLE 9.—*Electrometric titrations of sodium carbonate and of sodium hydroxide–sodium carbonate solutions*

[To 25 c. c. portions of solution of normality indicated various amounts of normal hydrochloric acid were added, and mixtures were made up to 50 c. c. volumes with distilled water]

| 1.0 normal $Na_2CO_3$ solution | | 1.0 normal solution containing NaOH (0.5 normal) and $Na_2CO_3$ (0.5 normal) | |
|---|---|---|---|
| Cubic-centimeters of 1.0 normal HCl added | pH | Cubic-centimeters of 1.0 normal HCl added | pH |
| 0 | 11.30 | 0 | 13.11 |
| 2 | 10.40 | 2 | 13.01 |
| 4 | 10.09 | 4 | 12.93 |
| 6 | 9.72 | 6 | 12.86 |
| 8 | 9.44 | 8 | 12.68 |
| 10 | 9.08 | 10 | 12.42 |
| 12 | 8.49 | 12 | 11.39 |
| 12.5 | 8.33 | 12.5 | 10.82 |
| 13 | 8.19 | 14 | 10.05 |
| 14 | 8.10 | 16 | 9.52 |
| 16 | 7.84 | 18 | 8.77 |
| 18 | 7.53 | 20 | 7.73 |
| 22 | 7.14 | 22 | 7.47 |
| 24 | 6.74 | 24 | 7.30 |
| 24.5 | 6.66 | 24.5 | 7.08 |
| 25 | 3.73 | 25 | 4.57 |

### BUFFER INDEX

It is possible to determine at any point on the curves shown in these figures the rate at which the pH changes with addition of acid. For the purpose of this work it was thought satisfactory to determine the amount of acid necessary to give a change in hydrogen-ion concentration of 0.5 pH, this being measured from the point selected on the curve. We may represent this by $\frac{d \text{ acid}}{d \text{ pH}}$ where $d$ pH = 0.5. This quantity, then, is the buffer index for the mixture at that point on the curve.

### OSMOTIC PRESSURE

The osmotic pressure was calculated from the freezing-point depression obtained with the conventional Beckmann apparatus, for those solutions from which the solute did not precipitate at the freezing point. The phosphate solutions were in most cases so concentrated that precipitation occurred before the freezing point was reached. For these solutions it was necessary to resort to the more troublesome method of determining the boiling point.

The method used for the boiling-point determinations was essentially that described by Bigelow (*5*). The apparatus consisted of an unsilvered Dewar flask mounted in a wood block. An inverted funnel, the stem of which was about 1 cm. in diameter and contained projections similar to a Vigreux tube, was suspended a short distance from the bottom of the flask by means of the glass tubes that carried the heating wires. In the ends of these tubes were fused short pieces of platinum wire. To these leads was attached the heating coil (platinum wire of No. 32 gage). The heating coil was placed just beneath

the inverted funnel. A 110-volt alternating current, after passing through a suitable constant resistance, was passed through the heating coil. The Beckmann thermometer was inserted through the stopper into the tube leading from the inverted funnel, the lower end of the Beckmann remaining slightly above the surface of the liquid. During the boiling, the liquid and vapor circulated past the bulb of the thermometer. A return condenser was fitted in the stopper, the end of the condenser tube being curved so as to come in contact with the side of the flask and thus prevent cold water from dropping near the thermometer. The room temperature was kept at 25° C. during the time of making the final reading. The barometric pressure was carefully read every few minutes while the final readings were being made.

The molar freezing-point constant used was 1.85° C.; the boiling-point constant was 0.52°; and the factor used to correct for the influence of changes in barometric pressure on the boiling point was 0.0376° for each 1-mm. change in barometric pressure. The osmotic pressure of a molar solution was taken as 22.4 atmospheres at 0°.

The data obtained are recorded in Table 10. Since the osmotic pressure values were used only for comparison of the solutions, they were calculated for 0° C. The osmotic pressure at 60°, the temperature at which the germicidal properties were determined, can be obtained approximately by multiplying the values in Table 10 by $\frac{333}{273}$ or 1.22.

TABLE 10.—*Osmotic pressures of solutions as calculated for 0° C. from freezing point depressions and boiling point elevations*

| Solution No. | Alkali | Normality | Percentage of alkali neutralized | Freezing-point depression (°C.) | Osmotic pressure in atmospheres | Boiling-point elevation (°C.) | Osmotic pressure in atmospheres |
|---|---|---|---|---|---|---|---|
| 1 | NaOH | 0.5 | 0 | 1.748 | 21.2 | | |
| 2 | do | .5 | 50 | 1.766 | 21.4 | | |
| 3 | do | .25 | 0 | .871 | 10.6 | | |
| 4 | NaOH–$Na_2CO_3$ | .5 | 0 | 1.419 | 17.2 | | |
| 5 | NaOH–$Na_3PO_4$ | .5 | 0 | | | 0.433 | 18.7 |
| 6 | $Na_3PO_4$ | .5 | 0 | | | .429 | 18.5 |
| 7 | NaOH–$Na_2CO_3$ | .5 | 42 | 1.399 | 16.9 | | |
| 8 | NaOH–$Na_3PO_4$ | .5 | 50 | | | .476 | 20.5 |
| 9 | NaOH | .5 | 92 | 1.734 | 21.0 | .483 | 20.8 |
| 10 | do | .04 | 0 | .161 | 2.0 | | |
| 11 | $Na_2CO_3$ | .5 | 0 | 1.049 | 12.7 | | |
| 12 | $Na_3PO_4$ | .5 | 24 | | | .503 | 21.7 |
| 13 | NaOH–$Na_2CO_3$ | .5 | 48 | 1.429 | 17.3 | | |
| 14 | NaOH–$Na_3PO_4$ | .5 | 64 | | | .495 | 21.3 |
| 15 | NaOH | .5 | 97.1 | 1.717 | 20.8 | | |
| 16 | $Na_3PO_4$ | .5 | 50 | | | .591 | 25.5 |
| 17 | NaCl | .1455 | 0 | .519 | 6.3 | | |
| 18 | do | .5 | 0 | 1.716 | 21.4 | .488 | 21.0 |

Both freezing-point and boiling-point determinations were made with a few solutions for the purpose of comparing the results obtained by the two methods. The osmotic pressures recorded in other places in this paper are calculated on the basis of the freezing-point determinations, except in the case of solutions containing phosphates. For all solutions containing phosphates osmotic pressures are calculated from boiling-point elevations.

### The Test Organism and the Preparation and Storage of Spore Suspensions

The test organism used was a spore-forming aerobe obtained through the courtesy of Max Levine, of the Iowa State College. According to his records, this was culture No. 25, and it will be so referred to in this paper. Levine has used this organism in similar experiments to determine the germicidal properties of alkaline solutions, and has described it (Levine, Buchanan, and Lease (*10*)) as resembling *Bacillus laterosporous* and *B. ruminatus* described by Bergey (*3*). The only characteristic mentioned by Levine, Buchanan, and Lease that could not be confirmed was the production of indol. The writer found that the organism did not form indol.

After the colonies of culture No. 25 had been observed on several agar plates, a typical colony was picked, and the culture started from this colony was used in the investigation. This culture is referred to as No. 25 to avoid confusion, though it is realized that picking a single colony might change the characteristics of the culture. Standard nutrient agar was used as the culture medium.

About 30 c. c. of melted agar were transferred to 200 c. c. dilution bottles. After sterilization, the bottles were laid flat till the agar solidified. One cubic centimeter of a diluted 24-hour broth culture was used to inoculate the surface of the agar in these bottles. After incubation of these cultures for 14 days at 37° C., the growth was scraped and washed off with 0.85 per cent sodium chloride, and a suspension prepared. The sodium chloride solution was kept cold during this process in order to prevent any tendency of the spores to germinate. The material scraped from the agar was washed three times with 0.85 per cent sodium chloride by means of a high-speed centrifuge. A suspension was again made of the organisms and filtered through sterile gauze and then tubed. The tubes were heated in a water bath at 80° C. for 10 minutes to kill any vegetative forms that were present. They were then cooled, the cotton plugs cut off even with the top of the tube, and sealed with paraffin. These tubes of spore suspension were then placed in storage in a room kept at temperatures between −18° and −10° C. The tubes were put in storage on October 3, 1927. The spores, as is shown in the data presented below, changed very little, if any, in resistance during the period of storage.[3]

### Germicidal Tests

Various methods have been used to measure the germicidal strength of a disinfectant. Methods that determine the time required to kill all the bacteria in a given solution obviously are subject to greater error than those that determine rates. This latter type of method, by showing the course of disinfection, also gives some information on the mechanism of the process. In this study the end-point method was used only in preliminary work, the solutions later being tested by counting the survivors at various intervals by means of agar plates. In the end-point method, however, the procedure was somewhat different from that ordinarily followed. Pipettes were used to remove samples from the test mixture to tubes of nutrient broth, and dilutions were made by transferring from tube to tube a definite volume (0.1 c. c.). A typical chart appeared as follows:

[3] It was at the suggestion of Otto Rahn, of the department of dairy industry, that freezing the spore suspensions was adopted as a method of preserving the viability of the spores.

| Time of exposure—minutes | Dilution of test solution in broth tubes | | | |
|---|---|---|---|---|
| | 1/10² | 1/10³ | 1/10⁴ | 1/10⁵ |
| 4 | + | + | + | + |
| 8 | + | + | + | + |
| 12 | + | + | + | + |
| 16 | + | + | + | + |
| 20 | + | + | − | − |
| 24 | + | − | − | − |
| 28 | − | − | − | − |

+ indicates growth. − indicates no growth.

This method was valuable as a guide to follow in plating, and in many instances checked surprisingly well with the plate method, but was too often irregular.

For the main determinations the method used was to enumerate the surviving spores at various intervals of time by plating on agar plates.

The water bath used was a small Freas water thermostat, the regular heating unit being supplemented with extra lamps amounting to 300 watts. The temperature variation was always within ±0.5° C., and rarely and only for short periods reached this large value. The usual variation was ±0.1°.

The test solutions were put in 500 c. c. 3-necked Woulff bottles which were suspended in the water bath. These bottles were fitted with stirring devices belted up to the motor operating the main stirrer for the water bath. Two of the openings of the test bottle were stoppered with cotton, while the middle opening was fitted with a cork through which the stirring rod passed. The bottle fitted with the stirrer was sterilized in the hot-air oven. Since stock solutions of normal alkali and acid were used, sterilization was unnecessary when the solutions were measured out aseptically. It was found by running blanks that sterile solutions could be prepared by measuring out the acid and alkali aseptically and by using sterile water.

The amount of solution for the test was 200 c. c., made up of such a strength that when 5 c. c. of the solution were removed for a blank, and 5 c. c. of spore suspension added, the solution would be of the desired normality. The spore suspension was thus diluted 40 times by adding 5 c. c. to 195 c. c. of the solution.

Immediately before the experiment the frozen spore suspension was warmed to near room temperature and filtered through a sterile coarse filter paper. The spore suspension was thoroughly shaken and was plated out in duplicate dilutions with duplicate plates, and sometimes with triplicate plates. In computing the number of viable spores present in the test solution at zero time, one-fortieth of the count of the spore suspension was taken. At given intervals 5 c. c. of the test mixture were removed and added to 45 c. c. sterile water blanks containing a sufficient amount of acid to neutralize

the alkali present. This neutralization was checked in each experiment by adding methyl orange indicator. This dilution was then plated out and higher dilutions made and plated out on standard nutrient agar. The plates were incubated at 37° C. for 48 hours and counted. The counts were recorded as number of colonies per cubic centimeter of the test solution.

## RESULTS

The results of the experiments on the germicidal action of the various mixtures are recorded in Tables 11 to 28. In each table the description of the solution is given. The normality of the solution with respect to total alkalinity is stated, and if hydrochloric acid was added the percentage of total alkalinity neutralized is shown. The pH of the solution at 25° C., the buffer index at 25°, and the osmotic pressure of the solution at 0° are in each instance recorded.

TABLE 11.—*Death rate of spores of culture No. 25 in 0.5 normal NaOH at 60° C.*

[Solution 1: pH, 13.36; buffer index, 37.6; osmotic pressure, 21.2 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Oct. 15, 1927 | 0 | 215,000 | 100.0 | 2.000 |
| | 2 | 95,000 | 44.2 | 1.645 |
| | 4 | 24,400 | 11.4 | 1.057 |
| | 6 | 2,650 | 1.23 | .090 |
| | 8 | 90 | .042 | 2.623 |
| | 10 | 0 | .0 | |
| | pH at end of experiment, 13.29 | | | |
| Nov. 25, 1927 | 0 | 102,000 | 100.0 | 2.000 |
| | 2 | 56,000 | 56.7 | 1.754 |
| | 4 | 27,900 | 27.3 | 1.436 |
| | 6 | 14,000 | 13.7 | 1.137 |
| | 8 | 260 | .254 | 1.405 |
| | 10 | 0 | | |
| | pH at end of experiment, 13.29 | | | |
| Jan. 10, 1928 | 0 | 245,000 | 100.0 | 2.000 |
| | 2 | 132,000 | 53.9 | 1.713 |
| | 4 | 65,000 | 26.5 | 1.405 |
| | 6 | 11,600 | 4.73 | .657 |
| | 8 | 2,710 | 1.11 | .025 |
| | 10 | 0 | .0 | |
| | pH at end of experiment, 13.32 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 2 | 1.68 | 0.32 | 1.152 | 0.369 |
| 4 | 1.29 | .71 | .576 | .409 |
| 6 | .61 | 1.39 | .384 | .534 |
| 8 | 1.35 | 2.65 | .288 | .764 |

Time required to kill 99 per cent of spores, 7.1 minutes.

TABLE 12.—*Death rate of spores of culture No. 25 in 0.5 normal NaOH (50 per cent neutralized) at 60° C.*

[Solution 2: pH, 13.10; buffer index, 17.0; osmotic pressure, 21.4 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Oct. 31, 1927 | 0 | 447,000 | 100.0 | 2.000 |
| | 2 | 306,000 | 68.5 | 1.836 |
| | 4 | 245,000 | 54.8 | 1.739 |
| | 8 | 159,000 | 35.5 | 1.550 |
| | 12 | 71,000 | 15.9 | 1.201 |
| | 16 | 11,500 | 2.57 | .410 |
| | 20 | 180 | .040 | $\bar{2}$.602 |
| | pH at end of experiment, 13.04 | | | |
| Feb. 15, 1928 | 0 | 142,000 | 100.0 | 2.000 |
| | 3 | 79,000 | 55.5 | 1.744 |
| | 6 | 56,000 | 39.3 | 1.594 |
| | 8 | 39,000 | 27.4 | 1.438 |
| | 12 | 13,300 | 9.34 | .970 |
| | 16 | 340 | .239 | $\bar{1}$.378 |
| | 20 | 40 | .0281 | $\bar{2}$.449 |
| | pH at end of experiment, 13.03 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | 2.303/$t$ | $K$ |
|---|---|---|---|---|
| 4 | 1.76 | 0.24 | 0.576 | 0.138 |
| 8 | 1.48 | .52 | .288 | .150 |
| 12 | 1.00 | 1.00 | .192 | .192 |
| 16 | .08 | 1.92 | .144 | .276 |
| 20 | $\bar{2}$.53 | 3.47 | .115 | .399 |

Time required to kill 99 per cent of spores, 16.2 minutes.

TABLE 13.—*Death rate of spores of culture No. 25 in 0.25 normal NaOH at 60° C.*

[Solution 3: pH, 13.12; buffer index, 17.0; osmotic pressure, 10.6 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Dec. 3, 1927 | 0 | 350,000 | 100.0 | 2.000 |
| | 4 | 381,000 | 108.9 | 2.037 |
| | 8 | 181,000 | 51.7 | 1.714 |
| | 12 | 88,000 | 25.2 | 1.401 |
| | 16 | 24,700 | 7.06 | .849 |
| | 20 | 15,600 | 4.46 | .649 |
| | 24 | 3,150 | .900 | $\bar{1}$.954 |
| | 28 | 130 | .037 | $\bar{2}$.568 |
| | 32 | 40 | .011 | $\bar{2}$.041 |
| | pH at end of experiment, 13.08 | | | |
| Feb. 27, 1928 | 0 | 260,000 | 100.0 | 2.000 |
| | 4 | 147,000 | 56.5 | 1.752 |
| | 8 | 119,000 | 45.8 | 1.661 |
| | 16 | 75,000 | 28.8 | 1.459 |
| | 20 | 32,300 | 12.4 | $\bar{1}$.093 |
| | 24 | 2,590 | .996 | $\bar{1}$.998 |
| | 28 | 840 | .323 | $\bar{1}$.509 |
| | 36 | 70 | .0269 | $\bar{2}$.430 |
| | pH at end of experiment, 13.10 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | 2.303/$t$ | $K$ |
|---|---|---|---|---|
| 4 | 1.87 | .013 | 0.576 | 0.0749 |
| 8 | 1.70 | .30 | .288 | .0864 |
| 16 | 1.18 | .82 | .144 | .1180 |
| 24 | .02 | 1.98 | .0960 | .1900 |
| 32 | $\bar{2}$.50 | 3.50 | .0720 | .2520 |

Time required to kill 99 per cent of spores, 24.0 minutes.

TABLE 14.—*Death rate of spores of culture No. 25 in 0.5 normal* $\frac{NaOH}{Na_2CO_3}$ *at 60° C.*

[Solution 4: pH, 13.11; buffer index, 17.0; osmotic pressure, 17.2 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Nov. 23, 1927 | 0 | 103,000 | 100.0 | 2.000 |
| | 2 | 73,000 | 70.9 | 1.848 |
| | 4 | 59,000 | 57.3 | 1.755 |
| | 6 | 61,000 | 59.2 | 1.769 |
| | 8 | 42,000 | 40.8 | 1.608 |
| | 10 | 21,300 | 20.7 | 1.314 |
| | 12 | 10,900 | 10.6 | 1.021 |
| | 14 | 6,800 | 6.60 | .817 |
| | 16 | 1,580 | 1.53 | .182 |
| | 20 | 50 | .048 | 2.681 |
| | pH at end of experiment, 13.10 | | | |
| Dec. 1, 1927 | 0 | 297,000 | 100.0 | 2.000 |
| | 2 | 226,000 | 76.1 | 1.880 |
| | 6 | 130,000 | 43.7 | 1.641 |
| | 8 | 85,000 | 28.6 | 1.456 |
| | 10 | 52,000 | 17.5 | 1.243 |
| | 12 | 25,900 | 8.71 | .940 |
| | 14 | 12,000 | 4.04 | .606 |
| | 16 | 2,780 | .935 | 1.971 |
| | 18 | 790 | .266 | 1.425 |
| | 20 | 190 | .064 | 2.806 |
| | pH at end of experiment, 13.12 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 4 | 1.80 | 0.20 | 0.576 | 0.115 |
| 8 | 1.52 | .48 | .288 | .138 |
| 12 | 1.00 | 1.00 | .192 | .192 |
| 16 | .10 | 1.90 | .144 | .274 |
| 20 | 2.75 | 3.25 | .115 | .374 |

Time required to kill 99 per cent of spores, 16.3 minutes.

TABLE 15.—*Death rate of spores of culture No. 25 in 0.5 normal* $\frac{NaOH}{Na_3PO_4}$ *at 60° C.*

[Solution 5: pH, 13.17; buffer index, 19.5; osmotic pressure, 18.7 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Nov. 15, 1927 | 0 | 165,000 | 100.0 | 2.000 |
| | 2 | 62,000 | 37.6 | 1.575 |
| | 4 | 37,000 | 22.4 | 1.350 |
| | 6 | 39,000 | 23.6 | 1.373 |
| | 8 | 16,800 | 10.2 | 1.009 |
| | 10 | 7,400 | 4.48 | .651 |
| | 12 | 1,850 | 1.12 | .049 |
| | pH at end of experiment, 13.08 | | | |
| Jan. 12, 1928 | 0 | 209,000 | 100.0 | 2.000 |
| | 2 | 139,000 | 66.4 | 1.822 |
| | 4 | 79,000 | 37.8 | 1.578 |
| | 6 | 61,000 | 29.2 | 1.465 |
| | 8 | 28,700 | 13.7 | 1.137 |
| | 10 | 4,800 | 2.30 | .362 |
| | 12 | 2,820 | 1.35 | .130 |
| | 14 | 160 | .0765 | 2.884 |
| | pH at end of experiment, 13.15 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 2 | 1.78 | 0.22 | 1.152 | 0.254 |
| 4 | 1.55 | .45 | .576 | .260 |
| 6 | 1.30 | .70 | .384 | .269 |
| 8 | 1.00 | 1.00 | .288 | .288 |
| 10 | .57 | 1.43 | .230 | .329 |
| 12 | 1.98 | 2.02 | .192 | .388 |
| 14 | 2.88 | 3.12 | .165 | .515 |

Time required to kill 99 per cent of spores, 12 minutes.

TABLE 16.—*Death rate of spores of culture No. 25 in 0.5 normal $Na_3PO_4$ at 60° C.*

[Solution 6: pH, 12.36: buffer index, 5.4; osmotic pressure, 18.5 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Nov. 23, 1927 | 0 | 104,000 | 100.0 | 2.000 |
| | 15 | 73,000 | 70.2 | 1.848 |
| | 30 | 45,000 | 43.2 | 1.638 |
| | 45 | 25,900 | 24.9 | 1.398 |
| | 60 | 8,800 | 8.5 | .929 |
| | 75 | 1,500 | 1.45 | .161 |
| | 90 | 280 | .270 | $\bar{1}$.431 |
| | 105 | 160 | .154 | $\bar{1}$.188 |
| | 120 | 40 | .039 | $\bar{2}$.591 |
| | pH at end of experiment, 12.31 | | | |
| Nov. 29, 1927 | 0 | 295,000 | 100.0 | 2.000 |
| | 15 | 166,000 | 56.3 | 1.751 |
| | 30 | 96,000 | 32.6 | 1.513 |
| | 45 | 56,000 | 19.0 | 1.279 |
| | 60 | 7,900 | 2.68 | .428 |
| | 75 | 780 | .264 | $\bar{1}$.422 |
| | 90 | 340 | .115 | $\bar{1}$.061 |
| | 105 | 120 | .0407 | $\bar{2}$.610 |
| | 120 | 30 | .0102 | $\bar{2}$.009 |
| | pH at end of experiment, 12.33 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 20 | 1.72 | 0.28 | 0.1152 | 0.0323 |
| 40 | 1.30 | .70 | .0576 | .0403 |
| 60 | .72 | 1.28 | .0384 | .0491 |
| 80 | $\bar{1}$.95 | 2.05 | .0288 | .0590 |
| 100 | $\bar{1}$.00 | 3.00 | .0230 | .0690 |

Time required to kill 99 per cent of spores, 79 minutes.

TABLE 17.—*Death rate of spores of culture No. 25 in 0.5 normal* $\frac{NaOH}{Na_2CO_3}$ *(42 per cent neutralized) at 60°C.*

[Solution 7: pH, 12.27; buffer index, 2.0; osmotic pressure, 16.9 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Dec. 7, 1927 | 0 | 350,000 | 100.0 | 2.000 |
| | 15 | 272,000 | 77.7 | 1.890 |
| | 30 | 178,000 | 50.8 | 1.706 |
| | 45 | 131,000 | 37.4 | 1.573 |
| | 60 | 102,000 | 29.2 | 1.465 |
| | 75 | 32,300 | 9.23 | .965 |
| | 90 | 16,400 | 4.69 | .671 |
| | 120 | 14,800 | 4.23 | .626 |
| | 150 | 9,200 | 2.63 | .420 |
| | 180 | 3,360 | .960 | $\bar{1}$.982 |
| | 210 | 1,250 | .357 | $\bar{1}$.553 |
| | pH at end of experiment, 12.27 | | | |
| Feb. 2, 1928 | 0 | 162,000 | 100.0 | 2.000 |
| | 15 | 79,000 | 48.6 | 1.687 |
| | 30 | 63,000 | 38.8 | 1.589 |
| | 60 | 58,000 | 35.7 | 1.553 |
| | 90 | 15,200 | 9.35 | .971 |
| | 120 | 5,300 | 3.26 | .513 |
| | 150 | 7,200 | 4.44 | .647 |
| | 180 | 960 | .591 | $\bar{1}$.772 |
| | 240 | 820 | .505 | $\bar{1}$.703 |
| | pH at end of experiment, 12.23 | | | |
| Mar. 1, 1928 | 0 | 445,000 | 100.0 | 2.000 |
| | 30 | 125,000 | 28.1 | 1.449 |
| | 60 | 73,000 | 16.4 | 1.215 |
| | 120 | 37,000 | 8.31 | .920 |
| | 180 | 6,000 | 1.35 | .130 |
| | 240 | 1,320 | .296 | $\bar{1}$.471 |
| | 300 | 190 | .0427 | $\bar{2}$.630 |
| | pH at end of experiment, 12.21 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | 2.303/$t$ | $K$ |
|---|---|---|---|---|
| 50 | 1.46 | 0.54 | 0.0461 | 0.0249 |
| 100 | .91 | 1.09 | .0230 | .0251 |
| 150 | .37 | 1.63 | .0154 | .0251 |
| 200 | $\bar{1}$.82 | 2.18 | .0115 | .0251 |
| 250 | $\bar{1}$.28 | 2.72 | .00921 | .0250 |
| 300 | $\bar{2}$.75 | 3.25 | .00768 | .0250 |

Time required to kill 99 per cent of spores, 184 minutes.

TABLE 18.—*Death rate of spores of culture No. 25 in 0.5 normal* $\frac{NaOH}{Na_3PO_4}$ *(50 per cent neutralized) at 60° C.*

[Solution 8: pH, 12.22; buffer index, 4.0; osmotic pressure, 20.5 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Dec. 6, 1927 | 0 | 227,000 | 100.0 | 2.000 |
| | 15 | 138,000 | 60.6 | 1.783 |
| | 30 | 82,000 | 36.1 | 1.558 |
| | 45 | 56,000 | 24.6 | 1.391 |
| | 60 | 38,100 | 16.8 | 1.225 |
| | 75 | 11,400 | 5.01 | .700 |
| | 90 | 6,000 | 2.64 | .422 |
| | 105 | 1,070 | .471 | $\bar{1}$.673 |
| | 120 | 440 | .194 | $\bar{1}$.288 |
| | 135 | 150 | .0659 | $\bar{2}$.819 |
| | 150 | 100 | .0440 | $\bar{2}$.644 |
| | 165 | 80 | .0352 | $\bar{2}$.547 |
| | pH at end of experiment, 12.20 | | | |
| Feb. 29, 1928 | 0 | 373,000 | 100.0 | 2.000 |
| | 15 | 350,000 | 93.9 | 1.973 |
| | 30 | 289,000 | 77.5 | 1.889 |
| | 60 | 85,000 | 22.8 | 1.358 |
| | 90 | 12,000 | 3.22 | .508 |
| | 120 | 2,380 | .638 | $\bar{1}$.805 |
| | 150 | 1,070 | .287 | $\bar{1}$.458 |
| | 180 | 140 | .0375 | $\bar{2}$.574 |
| | pH at end of experiment, 12.15 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 40 | 1.58 | 0.42 | 0.0576 | 0.0242 |
| 80 | .75 | 1.25 | .0288 | .0360 |
| 120 | $\bar{1}$.73 | 2.27 | .0192 | .0436 |
| 160 | $\bar{2}$.68 | 3.32 | .0144 | .0478 |

Time required to kill 99 per cent of spores, 110 minutes.

TABLE 19.—*Death rate of spores of culture No. 25 in 0.5 normal NaOH (92 per cent neutralized) at 60° C.*

[Solution 9: pH 12.22; buffer index, 2.1; osmotic pressure, 21.0 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Dec. 1, 1927 | 0 | 297,000 | 100.0 | 2.000 |
| | 15 | 259,000 | 87.2 | 1.941 |
| | 45 | 147,000 | 49.5 | 1.695 |
| | 60 | 137,000 | 46.2 | 1.665 |
| | 75 | 90,000 | 30.3 | 1.481 |
| | 90 | 64,000 | 21.6 | 1.335 |
| | 105 | 27,800 | 9.35 | .971 |
| | 120 | 18,400 | 6.20 | .792 |
| | 135 | 12,200 | 4.11 | .614 |
| | 150 | 7,600 | 2.56 | .408 |
| | 180 | 2,890 | .974 | $\bar{1}$.989 |
| | 210 | 2,110 | .710 | $\bar{1}$.851 |
| | 240 | 940 | .316 | $\bar{1}$.500 |
| | pH at end of experiment, 12.22 | | | |
| Feb. 8, 1928 | 0 | 105,000 | 100.0 | 2.000 |
| | 30 | 71,000 | 66.8 | 1.825 |
| | 60 | 30,800 | 29.0 | 1.462 |
| | 90 | 15,300 | 14.4 | 1.158 |
| | 120 | 3,770 | 3.54 | .549 |
| | 150 | 1,970 | 1.85 | .267 |
| | 180 | 810 | .762 | $\bar{1}$.882 |
| | 210 | 560 | .526 | $\bar{1}$.721 |
| | 240 | 210 | .197 | $\bar{1}$.295 |
| | pH at end of experiment, 12.16 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | 2.303/$t$ | $K$ |
|---|---|---|---|---|
| 50 | 1.65 | 0.35 | 0.0461 | 0.0162 |
| 100 | 1.05 | .95 | .0230 | .0218 |
| 150 | .41 | 1.59 | .0154 | .0245 |
| 200 | $\bar{1}$.77 | 2.23 | .0115 | .0256 |

Time required to kill 99 per cent of spores, 182 minutes.

TABLE 20.—*Death rate of spores of culture No. 25 in 0.04 normal NaOH at 60° C.*

[Solution 10: pH, 12.45; buffer index, 2.7; osmotic pressure, 1.95 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Dec. 3, 1927 | 0 | 350,000 | 100.0 | 2.000 |
| | 60 | 226,000 | 64.5 | 1.809 |
| | 120 | 117,000 | 33.4 | 1.524 |
| | 240 | 46,300 | 13.2 | 1.061 |
| | 360 | 250 | .072 | 2.857 |
| | 480 | 10 | .003 | 3.477 |
| | pH at end of experiment, 12.40 | | | |
| Feb. 27, 1928 | 0 | 260,000 | 100.0 | 2.000 |
| | 30 | 236,000 | 90.8 | 1.958 |
| | 60 | 167,000 | 64.2 | 1.808 |
| | 120 | 91,000 | 35.0 | 1.544 |
| | 240 | 26,000 | 10.0 | 1.000 |
| | 300 | 8,000 | 3.08 | .489 |
| | 360 | 890 | .342 | 1.534 |
| | 420 | 10 | .0038 | 3.584 |
| | pH at end of experiment, 12.45 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 60 | 1.82 | 0.18 | 0.0384 | 0.00691 |
| 120 | 1.59 | .41 | .0192 | .00787 |
| 180 | 1.32 | .68 | .0128 | .00870 |
| 240 | 1.00 | 1.00 | .00959 | .00959 |
| 300 | .45 | 1.55 | .00768 | .01190 |
| 360 | $\bar{1}$.23 | 2.77 | .00640 | .01772 |

Time required to kill 99 per cent of spores, 326 minutes.

TABLE 21.—*Death rate of spores of culture No. 25 in 0.5 normal $Na_2CO_3$ at 60° C.*

[Solution 11: pH, 11.39; buffer index, 1.4; osmotic pressure, 12.7 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Nov. 29, 1927 | 0 | 295,000 | 100.0 | 2.000 |
| | 30 | 226,000 | 76.6 | 1.884 |
| | 60 | 170,000 | 57.6 | 1.760 |
| | 120 | 132,000 | 44.7 | 1.650 |
| | 180 | 91,000 | 30.8 | 1.489 |
| | 240 | 61,000 | 20.7 | 1.316 |
| | 360 | 16,500 | 5.59 | .747 |
| | 480 | 2,040 | .692 | $\bar{1}$.840 |
| | 600 | 190 | .0644 | 2.809 |
| | 720 | 80 | .0271 | 2.433 |
| | pH at end of experiment, 11.27 | | | |
| Feb. 15, 1928 | 0 | 142,000 | 100.0 | 2.000 |
| | 30 | 82,000 | 57.6 | 1.760 |
| | 60 | 72,000 | 50.6 | 1.704 |
| | 120 | 50,000 | 35.1 | 1.545 |
| | 240 | 12,800 | 8.98 | .953 |
| | 360 | 4,200 | 2.95 | .470 |
| | 480 | 250 | .175 | $\bar{1}$.243 |
| | 600 | 150 | .105 | $\bar{1}$.021 |
| | pH at end of experiment, 11.27 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 120 | 1.62 | 0.38 | 0.01920 | 0.00730 |
| 240 | 1.12 | .88 | .00959 | .00844 |
| 360 | .52 | 1.48 | .00640 | .00946 |
| 480 | $\bar{1}$.76 | .224 | .00480 | .01075 |
| 600 | 2.92 | .308 | .00384 | .01182 |

Time required to kill 99 per cent of spores, 444 minutes.

TABLE 22.—*Death rate of spores of culture No. 25 in 0.5 normal $Na_3PO_4$ (24 per cent neutralized) at 60° C.*

[Solution 12: pH, 11.35; buffer index, 6.2; osmotic pressure, 21.7 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Feb. 1, 1928 | 0 | 160,000 | 100.0 | 2.000 |
| | 60 | 90,000 | 56.3 | 1.751 |
| | 120 | 51,000 | 31.9 | 1.504 |
| | 240 | 6,300 | 3.94 | .596 |
| | 360 | 650 | .406 | 1.609 |
| | 480 | 20 | .0125 | 2.097 |
| | pH at end of experiment, 11.24 | | | |
| Feb. 6, 1928 | 0 | 82,500 | 100.0 | 2.000 |
| | 60 | 39,000 | 47.3 | 1.675 |
| | 120 | 15,700 | 19.0 | 1.279 |
| | 240 | 830 | 1.01 | .004 |
| | 360 | 230 | .279 | .1446 |
| | 480 | 110 | .133 | 1.124 |
| | pH at end of experiment, 11.20 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 120 | 1.35 | 0.65 | 0.01920 | 0.0125 |
| 240 | .46 | 1.54 | .00959 | .0148 |
| 360 | 1.50 | 2.50 | .00640 | .0160 |
| 480 | 2.50 | 3.50 | .00480 | .0168 |

Time required to kill 99 per cent of spores, 300 minutes.

TABLE 23.—*Death rate of spores of culture No. 25 in 0.5 normal $\frac{NaOH}{Na_2CO_3}$ (48 per cent neutralized) at 60° C.*

[Solution 13: pH, 11.30; buffer index, 1.0; osmotic pressure, 17.3 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Feb. 4, 1928 | 0 | 185,000 | 100.0 | 2.000 |
| | 60 | 113,000 | 61.1 | 1.786 |
| | 120 | 130,000 | 70.3 | 1.847 |
| | 240 | 52,000 | 28.2 | 1.450 |
| | 360 | 9,600 | 5.20 | .716 |
| | 480 | 2,980 | 1.61 | .207 |
| | 600 | 390 | .211 | 1.324 |
| | 720 | 90 | .0487 | 2.688 |
| | pH at end of experiment, 11.13 | | | |
| Feb. 17, 1928 | 0 | 235,000 | 100.0 | 2.000 |
| | 60 | 212,000 | 90.5 | 1.957 |
| | 120 | 112,000 | 47.6 | 1.678 |
| | 240 | 66,000 | 28.1 | 1.449 |
| | 360 | 27,300 | 11.6 | 1.065 |
| | 480 | 8,500 | 3.62 | .559 |
| | 600 | 1,620 | .690 | 1.839 |
| | 720 | 410 | .175 | 1.243 |
| | 840 | 160 | .068 | 2.833 |
| | pH at end of experiment, 11.00 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 120 | 1.75 | 0.25 | 0.01920 | 0.00480 |
| 240 | 1.38 | .62 | .00959 | .00595 |
| 360 | .90 | 1.10 | .00640 | .00704 |
| 480 | .35 | 1.65 | .00480 | .00792 |
| 600 | 1.67 | 2.33 | .00384 | .00895 |
| 720 | 1.00 | 3.00 | .00320 | .00960 |

Time required to kill 99 per cent of spores, 540 minutes.

TABLE 24.—*Death rate of spores of culture No. 25 in 0.5 normal* $\frac{NaOH}{Na_3PO_4}$ *(64 per cent neutralized) at 60° C.*

[Solution 14: pH, 11.28; buffer index, 2.9; osmotic pressure, 21.3 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Feb. 3, 1928 | 0 | 273,000 | 100.0 | 2.000 |
| | 60 | 178,000 | 65.2 | 1.814 |
| | 120 | 109,000 | 39.9 | 1.601 |
| | 180 | 89,000 | 32.6 | 1.513 |
| | 240 | 42,000 | 15.4 | 1.188 |
| | 360 | 11,200 | 4.10 | .613 |
| | 480 | 2,270 | .832 | $\bar{1}$.920 |
| | 600 | 490 | .180 | $\bar{1}$.255 |
| | 720 | 110 | .0403 | $\bar{2}$.605 |
| | pH at end of experiment, 11.20 | | | |
| Feb. 4, 1928 | 0 | 185,000 | 100.0 | 2.000 |
| | 60 | 83,000 | 44.9 | 1.652 |
| | 120 | 91,000 | 49.2 | 1.692 |
| | 240 | 38,000 | 20.6 | 1.314 |
| | 360 | 9,100 | 4.92 | .692 |
| | 480 | 2,340 | 1.27 | .104 |
| | 600 | 450 | .244 | $\bar{1}$.387 |
| | 720 | 80 | .0433 | $\bar{2}$.637 |
| | pH at end of experiment, 11.19 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 120 | 1.62 | 0.38 | 0.01920 | 0.00730 |
| 240 | 1.22 | .78 | .00959 | .00748 |
| 360 | .65 | 1.35 | .00640 | .00864 |
| 480 | .00 | 2.00 | .00480 | .00960 |
| 600 | $\bar{1}$.32 | 2.68 | .00384 | .01030 |
| 720 | $\bar{2}$.61 | 3.39 | .00320 | .01086 |

Time required to kill 99 per cent of spores, 480 minutes.

TABLE 25.—*Death rate of spores of culture No. 25 in 0.5 normal NaOH (97.1 per cent neutralized) at 60° C.*

[Solution 15: pH, 11.40; buffer index, 0.4; osmotic pressure, 20.8 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Feb. 6, 1928 | 0 | 82,500 | 100.0 | 2.000 |
| | 60 | 40,000 | 48.5 | 1.686 |
| | 120 | 16,400 | 19.9 | 1.299 |
| | 180 | 15,900 | 19.3 | 1.286 |
| | 240 | 8,500 | 10.3 | 1.013 |
| | 360 | 2,365 | 2.87 | .458 |
| | 480 | 1,890 | 2.29 | .360 |
| | 600 | 770 | .934 | $\bar{1}$.970 |
| | 720 | 140 | .170 | $\bar{1}$.230 |
| | 840 | 20 | .0242 | $\bar{2}$.384 |
| | 960 | 0 | .000 | |
| | pH at end of experiment, 11.12 | | | |
| Feb. 3, 1928 | 0 | 273,000 | 100.0 | 2.000 |
| | 60 | 167,000 | 61.2 | 1.787 |
| | 120 | 136,000 | 49.8 | 1.697 |
| | 180 | 101,000 | 37.0 | 1.568 |
| | 240 | 84,000 | 30.8 | 1.489 |
| | 360 | 53,000 | 19.4 | 1.288 |
| | 480 | 17,900 | 6.55 | .816 |
| | 600 | 5,600 | 2.05 | .312 |
| | 720 | 1,190 | .436 | $\bar{1}$.640 |
| | 840 | 640 | .234 | $\bar{1}$.369 |
| | 960 | 130 | .0476 | $\bar{2}$.678 |
| | pH at end of experiment, 11.03 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 120 | 1.60 | 0.40 | 0.01920 | 0.00768 |
| 240 | 1.30 | .70 | .00959 | .00671 |
| 360 | .90 | 1.10 | .00640 | .00704 |
| 480 | .56 | 1.44 | .00480 | .00691 |
| 600 | .09 | 1.91 | .00384 | .00733 |
| 720 | $\bar{1}$.52 | 2.48 | .00320 | .00794 |
| 840 | $\bar{2}$.92 | 3.08 | .00274 | .00844 |
| 960 | $\bar{2}$.23 | 3.77 | .00240 | .00905 |

Time required to kill 99 per cent of spores, 618 minutes.

TABLE 26.—*Death rate of spores of culture No. 25 in 0.5 normal $Na_3PO_4$ (50 per cent neutralized) at 60° C.*

[Solution 16: pH, 9.10; buffer index, 0.3; osmotic pressure, 25.5 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Feb. 17, 1928 | 0 | 235,000 | 100.0 | 2.000 |
| | 360 | 122,000 | 51.9 | 1.715 |
| | 720 | 105,000 | 44.7 | 1.650 |
| | 1,440 | 51,000 | 21.7 | 1.337 |
| | 2,160 | 26,300 | 11.2 | 1.049 |
| | 2,880 | 14,900 | 6.35 | .803 |
| | 5,040 | 690 | .294 | $\bar{1}$.468 |
| | 5,760 | 60 | .0256 | $\bar{2}$.408 |
| | 6,480 | 0 | .000 | |
| | pH at end of experiment, 9.10 | | | |
| Feb. 23, 1928 | 0 | 315,000 | 100.0 | 2.000 |
| | 720 | 91,000 | 28.9 | 1.461 |
| | 1,440 | 56,000 | 17.8 | 1.250 |
| | 2,880 | 22,900 | 7.27 | .862 |
| | 4,320 | 9,800 | 3.12 | .494 |
| | 5,040 | 1,650 | .524 | $\bar{1}$.719 |
| | 5,760 | 90 | .0286 | $\bar{2}$.456 |
| | 6,480 | 0 | .000 | |
| | pH at end of experiment, 9.07 | | | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 1,000 | 1.47 | 0.53 | 0.002303 | 0.00122 |
| 2,000 | 1.10 | .90 | .001152 | .00104 |
| 3,000 | .77 | 1.23 | .000768 | .000945 |
| 4,000 | .30 | 1.70 | .000576 | .000980 |
| 5,000 | $\bar{1}$.60 | 2.40 | .000461 | .00111 |
| 6,000 | $\bar{2}$.00 | 4.00 | .000384 | .00154 |

Time required to kill 99 per cent of spores, 4,500 minutes.

TABLE 27.—*Death rate of spores of culture No. 25 in 0.1455 normal NaCl at 60° C.*

[Solution 17: pH, 7.5 (approximate); buffer index, 0.0; osmotic pressure, 6.3 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Jan. 3, 1928 | 0 | 130,000 | 100.0 | 2.000 |
| | 360 | 107,000 | 82.4 | 1.916 |
| | 720 | 70,000 | 53.9 | 1.732 |
| | 1,440 | 43,000 | 33.2 | 1.521 |
| | 2,160 | 24,500 | 18.9 | 1.277 |
| | 2,880 | 13,300 | 10.2 | 1.009 |
| | 3,600 | 6,200 | 4.78 | .679 |
| | 4,320 | 3,200 | 2.46 | .391 |
| | 5,760 | 400 | .308 | $\bar{1}$.489 |
| | 6,480 | 30 | .0231 | $\bar{2}$.364 |
| | 6,960 | 10 | .0077 | $\bar{3}$.887 |
| Jan. 22, 1928 | 0 | 52,000 | 100.0 | 2.000 |
| | 480 | 37,000 | 71.2 | 1.848 |
| | 1,200 | 35,000 | 67.3 | 1.824 |
| | 1,920 | 29,200 | 56.2 | 1.745 |
| | 2,640 | 12,700 | 24.4 | 1.384 |
| | 3,360 | 11,200 | 21.5 | 1.328 |
| | 4,080 | 5,200 | 9.99 | .999 |
| | 4,800 | 2,480 | 4.76 | .674 |
| | 5,520 | 1,890 | 3.63 | .556 |
| | 6,240 | 470 | .895 | $\bar{1}$.952 |
| | 6,960 | 120 | .228 | $\bar{1}$.358 |
| | 7,680 | 20 | .0381 | $\bar{2}$.581 |
| | 8,400 | 0 | .0 | |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 1,000 | 1.750 | 0.250 | 0.002303 | 0.000576 |
| 2,000 | 1.500 | .500 | .001152 | .000576 |
| 3,000 | 1.200 | .800 | .000768 | .000615 |
| 4,000 | .820 | 1.180 | .000576 | .000680 |
| 5,000 | .380 | 1.620 | .000461 | .000746 |
| 6,000 | $\bar{1}$.770 | 2.230 | .000384 | .000856 |
| 7,000 | $\bar{2}$.710 | 3.290 | .000329 | .001082 |

Time required to kill 99 per cent of spores, 5,650 minutes.

TABLE 28.—*Death rate of spores of culture No. 25 in 0.5 normal NaCl at 60° C.*

[Solution 18: pH, 7.5 (approximate); buffer index, 0.0; osmotic pressure, 21.4 atmospheres]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Jan. 10, 1928 | 0 | 245,000 | 100.0 | 2.000 |
| | 360 | 227,500 | 92.6 | 1.967 |
| | 720 | 143,000 | 58.3 | 1.766 |
| | 1,080 | 90,000 | 36.7 | 1.565 |
| | 1,800 | 53,000 | 21.6 | 1.335 |
| | 2,520 | 29,600 | 12.1 | 1.083 |
| | 3,240 | 21,300 | 8.68 | .939 |
| | 3,960 | 14,800 | 6.03 | .780 |
| | 4,680 | 7,900 | 3.22 | .508 |
| | 5,400 | 6,100 | 2.49 | .396 |
| | 6,120 | 2,900 | 1.18 | .072 |
| Jan. 22, 1928 | 0 | 52,500 | 100.0 | 2.000 |
| | 480 | 37,000 | 70.5 | 1.848 |
| | 1,200 | 20,000 | 38.1 | 1.581 |
| | 1,920 | 15,600 | 29.7 | 1.473 |
| | 3,360 | 6,400 | 12.2 | 1.086 |
| | 4,080 | 1,320 | 2.52 | .401 |
| | 4,800 | 570 | 1.09 | .037 |
| | 5,520 | 290 | .552 | 1.742 |
| | 6,240 | 20 | .0381 | 2.581 |
| | 6,960 | 0 | .0 | |
| Feb. 23, 1928 | 0 | 315,000 | 100.0 | 2.000 |
| | 720 | 132,000 | 41.9 | 1.622 |
| | 1,440 | 101,000 | 32.0 | 1.505 |
| | 2,880 | 46,000 | 14.6 | 1.164 |
| | 3,600 | 22,900 | 7.26 | .861 |
| | 4,320 | 19,200 | 6.10 | .785 |
| | 5,040 | 4,300 | 1.37 | .137 |
| | 5,760 | 2,460 | .781 | 1.893 |
| | 6,480 | 430 | .137 | 1.137 |
| | 7,920 | 250 | .0794 | 2.900 |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 1,000 | 1.70 | 0.30 | 0.002303 | 0.000691 |
| 2,000 | 1.39 | .61 | .001152 | .000703 |
| 3,000 | 1.09 | .91 | .000768 | .000699 |
| 4,000 | .73 | 1.27 | .000576 | .000731 |
| 5,000 | .28 | 1.72 | .000461 | .000792 |
| 6,000 | 1.77 | 2.23 | .000384 | .000855 |
| 7,000 | 2.83 | 3.17 | .000329 | .001042 |

Time required to kill 99 per cent of spores, 5,500 minutes.

The actual counts determined from duplicate plates made at various intervals are tabulated. These counts have also been calculated in percentage of the original number of spores still surviving at the various times. These data were plotted, the logarithm of percentage surviving on the ordinate, the time in minutes on the abscissa. The duplicate or triplicate experiments were plotted on the same chart and the best fitting curve drawn through these points. Each curve, then, was the average of at least two different experiments. From this curve the logarithms of the percentage of spores surviving at different times $t$ have been taken and recorded in the lower part of the tables under "log $B$." The original number of spores present is always represented by 100 per cent. "Log $A$," in each instance, is the logarithm of 100 or 2. Using the equation for the monomolecular reaction, the constant $K$ has been calculated.

$$K=\frac{2.303}{t}\cdot\log\frac{A}{B}$$

In this way are obtained the values for $K$, which show the rate of disinfection at the times $t$. Calculations of this type have been made so often in disinfection experiments that it seemed desirable to treat the data in this way. No great importance, however, is attributed to these "constants." The results show that in almost every case $K$ does not remain constant, but increases toward the end of the disinfection process.

From the curve for each solution was taken the time required to kill 99 per cent of the spores. This time, the writer believes, is the best measure of the true disinfecting power of the solution. If the time necessary to kill 99.9 per cent or 99.99 per cent of the spores is taken, the error is greater, since the shape of the curve is too much influenced by small counts where the experimental error is large.

In Table 29 a summary is made of the data presented in Tables 11 to 28.

A study of this table will reveal the influence of the three factors, hydroxyl-ion concentration, buffer index, and osmotic pressure.

TABLE 29.—*Summary of data from Tables 11 to 28*

| Solution No. | Solution | Normality | Percentage of alkali neutralized with HCl | From titration curve (pH) | Average alkalinity at end of experiments (pH) | Buffer index $d$ acid/$d$ pH | Osmotic pressure (atmospheres) | Time required to kill 99 per cent of spores (minutes) | Killing time × OH-ion concentration* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | NaOH | 0.5 | 0 | 13.36 | 13.30 | 37.6 | 21.2 | 7.1 | 1.6 |
| 1 | do | .5 | 50 | 13.10 | 13.04 | 17.0 | 21.4 | 16.2 | 2.5 |
| 3 | do | .25 | 0 | 13.12 | 13.09 | 17.0 | 10.6 | 24 | 3.1 |
| 4 | NaOH–$Na_2CO_3$ | .5 | 0 | 13.11 | 13.11 | 17.0 | 17.2 | 16.3 | 2.1 |
| 5 | NaOH–$Na_2PO_4$ | .5 | 0 | 13.17 | 13.12 | 19.5 | 18.7 | 12 | 1.7 |
| 6 | $Na_2PO_4$ | .5 | 0 | 12.36 | 12.32 | 5.4 | 18.5 | 79 | 1.7 |
| 7 | NaOH–$Na_2CO_3$ | .5 | 42 | 12.27 | 12.24 | 2.0 | 16.9 | 184 | 3.4 |
| 8 | NaOH–$Na_2PO_4$ | .5 | 50 | 12.22 | 12.18 | 4.0 | 20.5 | 110 | 1.8 |
| 9 | NaOH | .5 | 92 | 12.22 | 12.19 | 2.1 | 21.0 | 182 | 2.9 |
| 10 | do | .04 | 0 | 12.45 | 12.43 | 2.7 | 2.0 | 326 | 9.2 |
| 11 | $Na_2CO_3$ | .5 | 0 | 11.39 | 11.27 | 1.4 | 12.7 | 444 | .9 |
| 12 | $Na_2PO_4$ | .5 | 24 | 11.35 | 11.22 | 6.2 | 21.7 | 300 | .6 |
| 13 | NaOH–$Na_2CO_3$ | .5 | 48 | 11.30 | 11.07 | 1.0 | 17.3 | 540 | .9 |
| 14 | NaOH–$Na_2PO_4$ | .5 | 64 | 11.28 | 11.20 | 2.9 | 21.3 | 480 | .9 |
| 15 | NaOH | .5 | 97.1 | 11.40 | 11.08 | .4 | 20.8 | 618 | 1.2 |
| 16 | $Na_2PO_4$ | .5 | 50 | 9.10 | 9.09 | .3 | 25.5 | 4,500 | .06 |
| 17 | NaCl | .1455 | 0 | ---------- | 7.5 | .0 | 6.3 | 5,650 | ---------- |
| 18 | do | .5 | 0 | ---------- | 7.5 | .0 | 21.4 | 5,500 | ---------- |

*Based on the average of the pH at the beginning and at the end of the experiment.

## DISCUSSION OF RESULTS

### Effect of Hydroxyl-ion Concentration on Germicidal Action

In Table 29 the various solutions are arranged on the whole in the order of decreasing pH. A general survey of the table shows that where the pH is high the time required to kill 99 per cent of the spores is small. As we go down the list the pH decreases and the time increases to a large figure.

The concentration of total alkali (determined with methyl orange as indicator) is the same in solutions 1, 4, 5, 6, and 11. The germicidal power, however, varies tremendously. Solution 1, for instance, requires only 7.1 minutes to kill 99 per cent of the spores, while solution 11 requires 444 minutes. The other solutions lie between these two extremes. In this group of solutions, namely, 1, 4, 5, 6, and 11, which have the same concentration of total alkali, the germicidal power correlates very well with the hydroxyl-ion concentration of the solution, the products of the hydroxyl-ion concentration and the killing time being 1.6, 2.1, 1.7, 1.7, and 0.9, respectively.

Probably at least four factors are involved in the killing effect produced by the solutions shown in these experiments, namely, heat, hydroxyl-ion concentration, buffer index, and osmotic pressure.

The fact that relatively the product of the hydroxyl-ion concentration times the killing time varies so little between pH 11.4 and 13.36 indicates the major influence of the hydroxyl-ion concentration on the killing, modified in certain specific instances by the buffer index and osmotic pressure. As the pH of the solution becomes less, the effect of the heat, unaided by such things as hydroxyl ions, buffer index, or osmotic pressure, becomes more and more the predominating factor, so that one would expect the product of the hydroxyl-ion concentration times the killing time to become less. In solutions 16, 17, and 18 heat is the main killing agency.

If we examine these results in more detail we may observe the influence of hydroxyl-ion concentration alone when the buffer index and osmotic pressure are constant. Comparing solutions 6 and 12, we see that the buffer indexes are nearly the same, but are in favor of solution 12. The osmotic pressure is also slightly in favor of solution 12. The pH of solution 6 is 12.36 and that of solution 12 is 11.35, a difference of one unit in pH, which indicates that the hydroxyl-ion concentration of solution 6 is ten times that of solution 12. Solution 6 required 79 minutes, while solution 12 required 300 minutes to kill 99 per cent of the spores; that is, the former is four times as effective as the latter.

Another illustration of the effect of pH is shown by comparing solutions 8 and 12. Though the buffer index is considerably in favor of solution 12, and the osmotic pressure slightly in favor of it, solution 8, by virtue of its greater concentration of hydroxyl ions, is nearly three times as effective. In this case the concentration of hydroxyl ions of solution 8 is a little over seven times that of solution 12.

Another instance of the effect of pH is shown by comparing solutions 7 and 14. Though the buffer index and osmotic pressure give solution 14 an advantage, solution 7 is about two and one-half times as effective. The hydroxyl-ion concentration of solution 7 is about ten times that of solution 14. Again the effect of pH is evidenced in solutions 9 and 14. Solution 14 has an advantage by virtue of its

greater buffer index; yet because solution 9 has a pH of 12.22 and solution 14 has a pH of 11.28 the former is about two and one-half times as effective as the latter.

### Effect of Buffer Index on Germicidal Action

Buffer index influences the germicidal action to a marked degree, as may be observed by comparing solutions in which the pH and osmotic pressure are fairly near the same.

Solution 8, for example, which has a buffer index of 4.0, is considerably more effective than solution 9, which has a buffer index of only 2.1.

Comparing solution 8 with solution 7, we see that the greater buffer index of solution 8 reduces the time necessary to destroy 99 per cent of the spores. The osmotic pressure in this case also favors solution 8, but the difference is not great enough to explain the difference in germicidal action.

Solution 12, by virtue of its buffer index of 6.2 as compared with the buffer index of 1.0 of solution 13, shows a greater increase in efficiency than can be explained by the slight differences in pH and osmotic pressure. Solutions 12 and 15 form another pair showing the effect of high buffer index in increasing the effectiveness of a given hydroxyl-ion concentration.

Another example of the effect of buffer index is shown in the case of solutions 14 and 15. Solution 14 has a buffer index of 2.9; solution 15 has a buffer index of 0.4. In spite of the fact that solution 15 had a higher pH to start with, solution 14 appears to be more effective because of its higher buffer index. In this particular instance it might be argued that the pH is the factor responsible for the difference in germicidal action rather than buffer index as such. However, the writer is not inclined to attribute all the difference in germicidal action to the difference in the final pH of the two solutions. The other three cases cited above show relatively small differences in the final pH, yet exhibit differences in germicidal action due to the buffer index alone.

It is thought that the buffer index measures the ability of the solution to furnish a constant supply of hydroxyl ions at the surface of the cell where hydroxyl ions are being removed from solution. Though the pH as measured in the body of the solution appears to be the same, it is easy to think that the pH in the liquid immediately around the cell becomes lower in the solution having a lower buffer index than it would in a solution having a higher buffer index. The final result is due to the concentration of hydroxyl ions, but the measurement of the concentration of hydroxyl ions in the body of the solution does not measure the concentration of hydroxyl ions in the solution immediately in contact with the cell.

### Effect of Osmotic Pressure on Germicidal Action

That osmotic pressure affects the germicidal action of an alkaline solution may be observed by comparing several of the solutions in Table 29. Solution 2 has an osmotic pressure twice that of solution 3, the pH and buffer index of these solutions being the same. Solution 2, because of its greater osmotic pressure, is able to destroy 99 per cent of the spores originally present in 16.2 minutes, as compared with 24 minutes required by solution 3.

A similar result is seen in a comparison of solutions 4 and 3. Solution 4, by virtue of considerably greater osmotic pressure, is the more effective in the destruction of spores.

In solutions 7 and 10 an outstanding case of the effect of osmotic pressure is observed. Solution 10 has the advantage of having both a higher pH and a higher buffer index. It has a very low osmotic pressure, however. Solution 7, by virtue of an osmotic pressure more than eight times that of solution 10, is able to overcome the handicap in pH and buffer index and gives a killing time of 184 minutes as compared with 326 minutes required by solution 10.

Another example parallel to the one just cited is seen in solutions 9 and 10. Solution 9 is considerably more effective than solution 10 by reason of having an osmotic pressure more than ten times as great as that of solution 10. Solution 9 was lower in both pH and buffer index.

Falk (*9*) has pointed out the fact that bacteria, more than almost any other group of animal and plant life, exhibit indifference to changes in osmotic pressure. He has observed also that effects which are commonly ascribed to osmotic-pressure relations are probably due to specific ion or molecule influences.

It will be noted in Table 29 that solution 17, which is 0.85 per cent sodium chloride, has an osmotic pressure of 6.3. Solution 18, which is about 2.9 per cent sodium chloride, has an osmotic pressure of 21.4, or more than three times the osmotic pressure of solution 17. Both solutions give very nearly the same killing times—5,650 and 5,500 minutes. Though the solution with the highest osmotic pressure shows a slightly shorter killing time, the difference is too small to be significant. It would appear from this that the effect of osmotic pressure is negligible in neutral salt solution, and yet there is no doubt that high osmotic pressures accelerated the action of alkali solutions in killing spores. It seems from these facts that the probable explanation is that the ions and molecules whose presence is measured by osmotic pressure act as catalysts in accelerating the action of the hydroxyl ion. This explanation is not new. As has been mentioned above, Paul, Birstein, and Reuss (*20*), Norton and Hsu (*18*), and others have recognized the action of ions and molecules in catalyzing disinfectant reactions. Loeb's (*14*) explanation of the "salt effect" in the mechanism of the diffusion of electrolytes through membranes may have some application here.

### Effect of a Combination of Factors on Germicidal Action

By reference to Table 29 it is apparent that the combined effect of high hydroxyl-ion concentration, high buffer index, and high osmotic pressure results in the most efficient killing. Solution 1, which is the most effective of the solutions shown, has high values for each of the three factors. Solution 3 has a high pH and a fairly high buffer index, but only a moderately high osmotic pressure, still it is effective. Solution 16, though the osmotic pressure is 25.5, is little more effective than the sodium chloride solution, since it has a low pH supported only by a very low buffer index.

Without continuing a citation of individual examples, it seems apparent that the hydroxyl-ion concentration is by far the most important factor of the three, but that the effectiveness of the hydroxyl-ion concentration is markedly affected by buffer index especially, and by osmotic pressure to a less degree.

## GERMICIDAL ACTION OF HYPOCHLORITES

To compare the germicidal power of the alkali solutions studied above with that of hypochlorite solutions, several experiments were carried out.

A neutral sodium hypochlorite solution was made by the electrolysis of a sodium chloride solution (approximately 1 normal). After this treatment the solution contained about 0.5 per cent available chlorine.

The alkaline sodium hypochlorite solution used in these experiments was prepared from a commercial washing powder which is a combination of trisodium phosphate and sodium hypochlorite. A concentrated solution of this powder was prepared and then diluted as desired.

In determining the total alkalinity in the hypochlorite solutions the method used was essentially that of Wesener and Teller (*28*).

The determination of the pH of the hypochlorites was complicated by the presence of chlorine just as in the case of the determination of total alkalinity. In order to test the pH colorimetrically or electrometrically, removal of this strong oxidizing agent was necessary. This was done by adding N/10 sodium thiosulphate to the hypochlorite solution till a negative test was obtained, when a drop of solution was placed in a drop of acidified potassium iodide solution to which a little starch solution was added. Since the amount of sodium thiosulphate solution necessary was so small, it was thought that the dilution factor was negligible, particularly since only an approximate determination of pH was desired. Both electrometric and colorimetric methods were used in determining the pH.

In making tests on the hypochlorites the spore suspension described above (p. 533) was used. The technic employed in making the germicidal tests was the same as that already described, except that instead of neutralizing with acid the solution in which the spores were exposed, as was done with the alkali solutions, the sample was transferred to a 1 per cent peptone solution to make the first dilution. Blanks were run to determine whether the presence of the largest amount of chlorine added influenced the count when the 1 per cent peptone was used for the dilution blank. These dilutions to which the chlorine was added were allowed to stand 40 minutes before plating. Counts made on the spore suspension, using the regular water dilution blanks, and counts made on the spore suspension, using the 1 per cent peptone solution to which hypochlorite had been added, checked within the experimental error. Since 40 minutes was a longer time than was generally needed in making the germicidal tests to plate out the dilutions, it was concluded that the amount of hypochlorite which was added by the sample of the test solution did not influence the counts when 1 per cent peptone was used as the dilution medium.

The results of the tests on the two hypochlorite solutions are recorded in Tables 30 and 31.

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TABLE 30.—*Death rate of spores of culture No. 25 in neutral sodium hypochlorite solution at 60° C.*

[pH, 7.5 (approximately); available chlorine, 0.01 per cent; titratable alkalinity, 0.000 normal alkali]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Mar. 12, 1928 | 0 | 173,000 | 100.0 | 2.000 |
| | 2 | *100 | *.058 | *2.763 |
| Mar. 30, 1928 | 0 | 260,000 | 100.0 | 2.000 |
| | 1 | 10 | .004 | 3.602 |
| | 2 | 0 | .000 | ---------- |
| | 4 | 0 | .000 | ---------- |

Time required to kill 99 per cent of spores, less than 1 minute.

* Less than figure given.

TABLE 31.—*Death rate of spores of culture No. 25 in alkaline sodium hypochlorite solution at 60° C.*

[pH, 11.4; available chlorine, 0.01 per cent; titratable alkalinity, 0.027 normal alkali]

| Date | Time of exposure in minutes | Number of survivors per cubic centimeter | Percentage surviving | Logarithm of percentage surviving |
|---|---|---|---|---|
| Mar. 12, 1928 | 0 | 173,000 | 100.0 | 2.000 |
| | 2 | 130,000 | 75.2 | 1.876 |
| | 4 | 127,000 | 73.5 | 1.866 |
| | 8 | 107,000 | 61.9 | 1.792 |
| | 12 | 75,000 | 43.4 | 1.638 |
| | 16 | 55,000 | 31.8 | 1.502 |
| | 20 | 1,660 | .960 | 1.982 |
| | 24 | 70 | .041 | 2.613 |
| | 30 | 0 | .000 | ---------- |
| Mar. 30, 1928 | 0 | 260,000 | 100.0 | 2.000 |
| | 4 | 227,000 | 87.4 | 1.942 |
| | 8 | 198,000 | 76.2 | 1.882 |
| | 12 | 150,000 | 57.7 | 1.761 |
| | 16 | 103,000 | 39.6 | 1.598 |
| | 20 | 28,400 | 10.9 | 1.037 |
| | 24 | 1,530 | .589 | 1.770 |
| | 30 | 2 | .008 | ---------- |
| | 36 | 0 | .000 | ---------- |

EQUATION OF FIRST ORDER REACTION APPLIED TO DEATH RATE, CALCULATED FROM CURVE

| $t$ | Log $B$ | Log $A/B$ | $2.303/t$ | $K$ |
|---|---|---|---|---|
| 5 | 1.90 | 0.10 | 0.461 | 0.0461 |
| 10 | 1.79 | .21 | .230 | .0483 |
| 15 | 1.60 | .40 | .154 | .0616 |
| 20 | .70 | 1.30 | .115 | .150 |
| 25 | 2.35 | 3.65 | .092 | .336 |

Time required to kill 99 per cent of spores, 21.8 minutes.

Comparing the times required to kill 99 per cent of spores in the neutral sodium hypochlorite and in the alkaline sodium hypochlorite, it is apparent that the alkali in the latter solution retarded the action of the hypochlorite in destroying the spores. The available chlorine was the same at the start in both solutions. Tests showed that after several hours the available chlorine decreased more rapidly in the neutral solution than in the alkaline solution. However, since the times of exposure were so short, the available chlorine present can be considered constant.

Prucha (*22*) has recently studied the effect of alkalinity on the stability of hypochlorite solutions, both in the presence and in the absence of organic matter. In the absence of organic matter alkali tended to make the hypochlorite solution more stable. On the other hand, when milk was added to the hypochlorite solutions the available chlorine disappeared much more rapidly in the solution to which alkali had been added. This seems to confirm the theory of Wright [4] that alkalinity increases oxidation and acidity chlorination. In the neutral solution the chlorine combines with the milk proteins and remains in available form to some extent, while in the alkaline solution the chlorine is reduced to a form which is no longer available.

The retardation of the action of hypochlorite solutions by the presence of alkali was predicted on the basis of measurements of oxidation potentials by Rideal and Evans (*23*). As appears from Tables 30 and 31, the time required to destroy 99 per cent of spores in the neutral sodium hypochlorite solution was less than 1 minute, while the time required to accomplish the same thing in the alkaline hypochlorite solution was about 22 minutes, a fact which seems to confirm this prediction.

The main object of these experiments was to compare the effectiveness of the strong alkali solutions with hypochlorites at the same temperature; that is, 60° C. Neutral sodium hypochlorite with 100 parts per million of available chlorine was considerably more effective than the strongest alkaline solution tested, namely, the 0.5 normal sodium hydroxide solution. The alkaline hypochlorite was very similar in effectiveness to the 0.25 normal sodium hydroxide solution.

Wright (*30*) has called attention to the fact that acidity increases chlorination, while alkalinity increases oxidation of substances by hypochlorites. In later studies [5] he presented further evidence in confirmation of the above statement concerning the effect of acidity and alkalinity on the action of hypochlorites. He suggests on the basis of his results that the most effective use of hypochlorites will be obtained when the reaction of the solution is slightly acid. This suggestion is based on the theory that the germicidal effectiveness of hypochlorites is due in large part to the combination of chlorine with proteins of the bacterial cell. Baker (*2*) points out that, since substances of greater oxidizing potential—such as permanganate—are less effective than chlorine, the killing action of chlorine is not due to direct oxidation. He further points out that the oxidizing power of chlorine can be considerably reduced by combination with ammonium, with only a very slight reduction in sterilizing efficiency. Chlorine, he thinks, attacks the lipoid surface of the cell and forms there compounds which bring about the death of the organism.

## RATE OF HYDROLYSIS OF GELATIN IN ALKALINE SOLUTIONS AS INFLUENCED BY HYDROXYL-ION CONCENTRATION, BUFFER INDEX, AND OSMOTIC PRESSURE

That the death of bacteria brought about through the action of heat or of disinfectants is due to injury to the protoplasm has often been pointed out in disinfection studies. Denaturation of true proteins has been considered as being brought about by the same in-

[4] WRIGHT, N. C. THE ACTION OF HYPOCHLORITES ON AMINO-ACIDS AND PROTEINS. THE INFLUENCE OF ACIDITY AND ALKALINITY. (Unpublished.)
[5] WRIGHT, N. C. Op. cit.

fluences that cause the death of bacteria. The death of bacteria may be due to a purely chemical reaction or to a physical-chemical effect in changing the colloidal state of the cell contents.

In an attempt to find out more about the processes which cause the death of bacteria when exposed to hot alkaline solutions, the following study was made. It was thought that possibly the course of hydrolysis of a protein would parallel the course of the death curve and would in some degree explain the process of death. It was recognized from the start that bacteria in the vegetative form and even spores are killed long before a protein is decomposed in an appreciable amount. Though the analogy is not exact, it is thought that the hydrolysis of protein may measure a later stage in the same processes which cause the death of the bacterial cell.

With this in mind a brief study was made to determine whether pH, buffer index, and osmotic pressure influenced the rate of hydrolysis of gelatin in the same way that they influenced the rate of death of bacterial spores.

The same apparatus used in the germicidal tests described above (p. 534) was employed. A larger amount of solution was used and larger samples were drawn at definite intervals.

The procedure consisted in adding to the 360 c. c. of alkali solution to be tested 40 c. c. of a 10 per cent solution of ash-free gelatin. The 360 c. c. of the alkali solution was made up of such a strength that when diluted by the addition of the gelatin solution the normality would be that desired. In the tables the normality of this final solution is recorded.

The pH was determined at the beginning of the experiment and at various times thereafter. The osmotic pressure recorded in the table is that determined in the pure solution at the strength indicated. The buffer index was calculated from the titration curves presented in Figures 1 and 2.

The rate of hydrolysis was measured by the amount of nitrogen present in the filtrate after the unhydrolyzed gelatin had been precipitated with tungstic acid. The sample (25 c. c.) of the solution was withdrawn and added to 41 c. c. of a hydrochloric acid solution of sufficient strength to neutralize the alkali added. This neutral (neutral to methyl orange) solution was put in the ice box and left one hour. Three cubic centimeters of a 20 per cent sodium tungstate solution were then added and the solution thoroughly agitated. Six cubic centimeters of 2 normal sulphuric acid were added, the solution being agitated constantly. This gave a final volume of 75 c. c. This mixture was left standing for one hour and filtered through paper.

Five minutes after the gelatin had been added to the test solution, two samples were withdrawn. One of these was used for analyzing the total nitrogen present. Nine cubic centimeters of water instead of the precipitating reagents were added to the solution. The other sample was treated with the sodium tungstate and sulphuric acid and the filtrate analyzed. This made it possible to determine just how complete the precipitation was, since very little splitting of the protein took place in this short time, though in the stronger solutions there was an appreciable amount of decomposition even in five minutes.

As hydrolysis proceeded the precipitation by the method described above became less efficient, and the only way found to overcome this was to allow the filtrate to stand 24 hours. Within a few minutes after filtration the filtrate became cloudy, and after it had stood for several hours the precipitate settled out.

The clear filtrate was carefully pipetted from the flask and analyzed for total nitrogen by the Kjeldahl method. A 50 c. c. sample of the filtrate was taken. Since the original 25 c. c. sample of the test solution was made up to a volume of 75 c. c., and 50 c. c. of the filtrate were taken, the total nitrogen found by analysis was the number of milligrams of nitrogen in 16⅔ c. c. of the test solution.

## RESULTS

The results of these experiments have been summarized in Tables 32 and 33. Two experiments were run with each solution and the data plotted, milligrams of nitrogen against time. The best fitting curve was drawn through these points. From this curve, the average of two experiments, were taken the amounts of soluble nitrogen at different times.

TABLE 32.—*The influence of osmotic pressure on the rate of hydrolysis of gelatin as shown in two solutions having the same pH and buffer index but different osmotic pressures*

| Time of exposure in hours | Milligrams of soluble nitrogen in— | |
|---|---|---|
| | Solution 3 (osmotic pressure, 10.6 atmospheres) | Solution 2 (osmotic pressure, 21.4 atmospheres) |
| 0 | 0.4 | 0.4 |
| 4 | 9.5 | 9.3 |
| 8 | 13.1 | 12.8 |
| 12 | 15.1 | 14.8 |
| 16 | 16.3 | 16.3 |
| 20 | 17.0 | 17.2 |
| 24 | 17.6 | 17.9 |
| 30 | 18.2 | 18.6 |

TABLE 33.—*The influence of buffer index on the rate of hydrolysis of gelatin as shown in two solutions having the same pH and osmotic pressure but different buffer indexes*

| Time of exposure in hours | Milligrams of soluble nitrogen in— | |
|---|---|---|
| | Solution 8 (buffer index 4.0) | Solution 9 (buffer index, 2.1) |
| 0 | 0.4 | 0.3 |
| 10 | 2.4 | 1.9 |
| 20 | 4.3 | 3.5 |
| 30 | 6.2 | 4.9 |
| 40 | 7.9 | 6.2 |
| 50 | 9.1 | 7.5 |
| 60 | 10.2 | 8.7 |
| 70 | 11.2 | 9.6 |
| 80 | 11.9 | 10.4 |

In Table 32 a comparison is made of the rates of hydrolysis in solutions 3 and 2. These two solutions have the same pH and the same buffer index, but solution 3 has an osmotic pressure of only 10.6 atmospheres, while solution 2 has an osmotic pressure of 21.4 atmospheres. The difference in the rate of hydrolysis is too small to be significant. It is concluded from this that the change in osmotic pressure in the range studied has no appreciable effect on the rate of hydrolysis. As has been shown above, a definite effect of osmotic pressure on germicidal action was observed, but this effect was relatively small.

In Table 33 is given a comparison of the rates of hydrolysis in solutions 8 and 9. These solutions have the same pH and approximately the same osmotic pressure, but solution 8 has a buffer index of 4.0, while that of solution 9 is only 2.1.

Though the difference in rates is small, it is believed that this difference is significant. After 80 hours' exposure, 11.9 mgm. of soluble nitrogen were formed in solution 8, while only 10.4 mgm. were formed in solution 9. It is concluded from this that the rate of hydrolysis of gelatin in alkali solutions is affected by the buffer index. The solution having the higher buffer index is the more effective.

The effect of pH and buffer index together is evident when a comparison of Tables 32 and 33 is made. Comparing solutions 2 and 8, it is seen that the higher pH, reinforced by a high buffer index, makes solution 2 very much more effective in hydrolyzing gelatin than solution 8. Solution 2 after 30 hours, for instance, gives 18.6 mgm. soluble nitrogen, while solution 8 gave only 6.2 mgm. It is apparent from these results that high buffer index and high pH favor the hydrolysis of gelatin. As has been pointed out above, Carpenter (*6*) found that the higher the hydroxyl-ion concentration the more rapid was the hydrolytic scission of casein.

Buffer index and pH, then, affect the hydrolysis of gelatin in the same way that they affected the killing of spores. The failure of osmotic pressure to influence the hydrolysis of gelatin in the same way that it influenced germicidal action may have been due to the existence of the cell wall of the spore. It seems probable that some of the ions and molecules which are responsible for the osmotic pressure may produce an effect only on the cell wall—possibly increasing its permeability for hydroxyl ions, for instance.

## GENERAL DISCUSSION AND PRACTICAL APPLICATION OF RESULTS

From the data presented it appears that the lowest pH that can be relied upon to make any given washing solution effective against *Bacterium coli* is pH 12.0. Even at room temperature solutions of this pH were able to destroy this organism within five minutes. Lower pH values were effective when the temperature was increased, and a given hydroxyl-ion concentration was rendered more effective by a high buffer index and a high osmotic pressure, the buffer-index factor appearing to be of considerably more importance than the osmotic-pressure factor. So if we have a pH of 12 in a solution which also has a high buffer value and high osmotic pressure, there is a good margin of safety. If, in actual practice, milk bottles are left in an alkali solution longer than five minutes, the margin of safety would be increased.

One disease-producing organism *Mycobacterium tuberculosis*, is remarkably resistant to strongly alkaline solutions, but is sensitive to heat. So to make certain that the washing solution at pH 12 will destroy this organism, a temperature of at least 140° F. (60° C.) should be maintained.

Now if we have an alkali solution which has a pH near 12 or higher, and which has a high buffer index (10 or higher), and maintain this solution at 60° C. or higher, we may be certain, from the information now at hand, that this solution will destroy in five minutes all the disease-producing organisms that might be encountered on a milk bottle, and leave a good margin of safety. A good margin of safety is required, too, because laboratory tests where homogeneous suspensions of bacteria are put in alkali solutions at exact temperatures show more effective killing than will tests made on solutions used in commercial practice where bacteria may be protected by films of dirt and where temperatures may vary.

If the solution just mentioned be accepted as satisfactory for use in the bottle-washing machine, the next step is to find a way to measure its condition at any time while it is in use and see whether it is still effective. Recontamination of the bottles with rinse water is excluded in this discussion, since water supplies are checked up in other ways.

By referring to Figures 1 and 2 and keeping in mind the results on germicidal action, we may obtain the information needed to develop a method of titration for the control of the effectiveness of the washing solution. A high pH and a high buffer index are desired (if the buffer index is high the osmotic pressure will of necessity be of considerable magnitude). From the curves in Figures 1 and 2 it can be seen that in the titrating of an alkali solution, when an indicator with an end point near pH 12.0, is used the indicator will show whether the pH of the solution is higher than 12, and the amount of the titration will give information as to the magnitude of the buffer index. Such a titration can be made with the aid of the indicator trinitrobenzene which in solutions above pH 12.0 has a deep orange color. The end point is somewhat indistinct and some color persists until 11.5 is reached, when the solution becomes colorless. If the titration is made fairly rapidly it works very well for approximate estimations. It can be seen from Figure 1 that such a titration would show most of the sodium hydroxide, a small part of the trisodium phosphate, and none of the sodium carbonate, were all of these present in solution.

Since the solution becomes colorless when the end point of trinitrobenzene is reached, phenolphthalein may then be added and the titration continued to the end point of this indicator. The carbonate and part of the first sodium of trisodium phosphate will appear in this titration. Since the solution again becomes colorless when the phenolphthalein end point is exceeded, methyl orange may next be added and the titration continued to determine the amounts of bicarbonate and of the secondary phosphate present. At the end point with trinitrobenzene we have data showing what may be called the "effective alkalinity," and the titrations with the other indicators give some idea of the amounts of the other constituents which contribute to the total alkalinity.

Table 34 contains data on the titrations of alkalies and alkali mixtures by the method just described. Table 35 shows the results of titrating some solutions of commercial washing powders.

TABLE 34.—*Data on titrations of alkalies and alkali mixtures*

| Alkali solutions used expressed in cubic centimeters of a normal solution | | | Normal HCl added to solution of mixture to obtain end point with— | | | | |
|---|---|---|---|---|---|---|---|
| | | | Trinitrobenzene | Phenolphthalein | | Methyl orange | |
| Normal NaOH | Normal $Na_2CO_3$ | Normal $Na_3PO_4$ | Total | Total | Increase above previous titration | Total | Increase above previous titration |
| *C. c.* | *C. c.* | *C. c.* | *C. c.* | *C. c.* | *C. c.* | *C. c.* | *C. c.* |
| 50 | | | 48.5 | 49.7 | 1.2 | 50 | 0.3 |
| 25 | 25 | | 22.8 | 37.7 | 14.9 | 50 | 12.3 |
| 25 | | 25 | 29.0 | 38.3 | 9.3 | 50 | 11.7 |
| | 50 | | .0 | 24.5 | 24.5 | 50 | 25.5 |
| | | 50 | 4.5 | 25.8 | 21.3 | 50 | 24.2 |

TABLE 35.—*Titration values of 5 per cent solutions of commercial washing powders*

[1 c. c. normal HCl, 0.04 gm. NaOH, 0.16 per cent NaOH, sample titrated, 25 c. c.]

| Powder | Normal HCl added to sample to obtain end point with— | | | | | |
|---|---|---|---|---|---|---|
| | Trinitrobenzene (effective alkalinity) | | Phenolphthalein | | Methyl orange | |
| | Total | NaOH | Total | Increase above previous titration | Total | Increase above previous titration |
| | *C. c.* | *Per cent* | *C. c.* | *C. c.* | *C. c.* | *C. c.* |
| A | 11.0 | 1.76 | 17.8 | 6.8 | 24.4 | 6.6 |
| B | 3.1 | .50 | 12.4 | 9.3 | 21.2 | 8.8 |
| C | .3 | .05 | 8.4 | 8.1 | 16.5 | 8.1 |
| E | .0 | .00 | 7.7 | 7.7 | 17.4 | 9.7 |
| F | .4 | .06 | 3.4 | 3.0 | 7.3 | 3.9 |
| X | 1.2 | .19 | 3.9 | 2.7 | 7.5 | 3.6 |

To state the exact amount that each titration should show in order for the solution to be effective, would be somewhat arbitrary. The following might be suggested, however, as safe minimum amounts. If the total alkalinity is 4 per per cent, for instance (this has proven satisfactory for the cleaning of bottles), 1 per cent should appear in the titration with trinitrobenzene. Three per cent then could be carbonates, phosphates, or other basic salts. Since the sodium hydroxide is neutralized first, it will have to be replenished more often than the carbonates or phosphates except where extremely "hard" water is used. The 1 per cent concentration for sodium hydroxide should be the minimum reached at any time, and of course when the washing solution is first made up the concentration should be much higher.

## SUMMARY

In this study tests were made on the germicidal properties of solutions of several commercial washing powders, with especial reference to the relations between the hydroxyl-ion concentration and germicidal efficiency.

Powders that gave solutions of high pH values were distinctly more effective as germicides than those that gave solutions of low pH values, the powder containing chlorine being an exception to this. Solutions that gave low pH values and accordingly weak germicidal action became more effective when the pH of the solution was increased by the addition of sodium hydroxide.

The germicidal efficiency of the washing powders used was not decreased to the same degree in each instance by dilution. One powder containing chlorine showed a very slight decrease in germicidal power when diluted considerably, while the other powders showed varying decreases in efficiency. Decreases in efficiency in these alkaline solutions appear to be closely correlated with decreases in hydroxyl-ion concentration when considered in reference to dilutions of the same powder.

Data on resistance of spores of *Bacillus cereus* show that a combination of high hydroxyl-ion concentration and high temperature is effective in destroying these spores within a few minutes, while either one alone is ineffective.

A study was made of the influence of the pH, buffer index, and osmotic pressure of alkaline solutions on their germicidal action at 60° C.

Spores of a spore-forming aerobe were used to measure germicidal action. Spore suspensions were prepared in 0.85 per cent sodium chloride solution, the vegetative forms were killed by exposure to heat, and the spore suspensions were stored in a frozen state. Over a period of six months no appreciable change in resistance was observed.

The death rate of spores in the alkaline solutions, with a few exceptions, did not follow the logarithmic rate but increased as disinfection proceeded. The death rate was increased by an increase in the hydroxyl-ion concentration of the solution when the buffer index and the osmotic pressure were kept constant. The death rate was increased by an increase in the buffer index of the solution when the pH and the osmotic pressure were kept constant. The death rate was increased by an increase in the osmotic pressure of the solution when the pH and the buffer index were kept constant. The osmotic-pressure factor had the least influence of the three factors studied.

A high pH supported by a high buffer index rendered a solution at a temperature of 60° C. extremely effective against spores.

The germicidal action of neutral sodium hypochlorite was compared with that of alkaline sodium hypochlorite, both solutions having the same amount of available chlorine. The neutral hypochlorite solution was by far the more effective. The alkaline hypochlorite solution with available chlorine present in 0.01 per cent concentration was similar in germicidal action to 0.25 normal sodium hydroxide solution.

A study was made of the influence of osmotic pressure, buffer index, and pH on the rate of hydrolysis of gelatin in strongly alkaline solutions. No appreciable effect of osmotic pressure on the rate of hydrolysis of gelatin was observed. The higher the buffer index and the higher the pH, the greater was the rate of hydrolysis of gelatin

A titration method has been proposed as a means of measuring quickly the germicidal potency of an alkaline washing solution. Data are given to illustrate how this method works on alkalies and alkali mixtures. The method consists in determining the amount of alkali that is capable of maintaining a pH above 12.0.

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# THE INFLUENCE OF SUBSTITUTED CATIONS ON THE PROPERTIES OF SOIL COLLOIDS[1]

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## INTRODUCTION

Several investigators have shown that the substitution of different monovalent and divalent bases in colloidal soil material markedly alters certain properties of the material. It has been found, for instance, that a colloidal soil material in which the exchangeable bases are replaced by sodium is more disperse, more viscous, and has a higher negative charge than the same material in which the bases are replaced by calcium or hydrogen. Little attention, however, has been given to the magnitudes of the effects produced in widely different kinds of colloidal soil material. This phase of the subject is of considerable importance in the study of soil colloids.

Data regarding the ranges of alteration producible in different colloidal soil materials should indicate in what measure certain properties of the materials are dependent on the kind of exchangeable base present. Previous studies in this bureau have shown that many properties of different colloidal soil materials vary approximately with the content of total exchangeable bases or with the ratio of silica to alumina and iron, suggesting that properties in general are governed in part by one or both of these features of chemical composition. It was expected, therefore, that even in the case of those properties which are markedly affected by the kind of exchangeable base, an influence of the $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio or of the content of total exchangeable bases would be apparent.

In this investigation seven colloidal materials which varied widely in properties and in ratio of $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ were studied. Determinations were made of the heat of wetting, absorption of water vapor, swelling, moisture equivalent, cataphoresis, and pH of these materials when saturated with Ca, Mg, K, Na, H, and the cation of methylene blue.

## REVIEW OF LITERATURE

The influence of the exchangeable cations Ca and Na upon the properties of the entire soil has been recognized for a long time. In 1879 Adolf Mayer (*21*)[2] showed that when soils are treated with NaCl and the excess of salt is washed out percolation is greatly retarded. Similar treatment with Ca $(OH)_2$ was found to produce very little change. In recent years many investigators, among them Sharp (*29*), Gedroiz (*9*), Hissink (*14*), Scofield and Headley (*28*), and Cummins and Kelly (*6*), have shown that saturation of the soil with the

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[2] Reference is made by number (italic) to "Literature cited," p. 583.

sodium ion markedly decreases percolation and increases deflocculation and swelling; whereas saturation with Ca tends to have an opposite effect. Recently Mattson (*20*) has shown a marked difference in the swelling of isolated colloids in which Na and Ca were substituted by treating the electrodialyzed material with NaOH and $Ca(OH)_2$.

The heat of wetting of isolated colloidal soil material as affected by saturation with different bases has been studied by Pate (*25*). Colloidal materials isolated from five soils all showed a slightly increased heat of wetting when the exchangeable bases present were substituted by Ca and a somewhat lower heat of wetting when $NH_4$ was substituted. The heats of wetting of six whole soils saturated with different bases were also determined. Substitution of Ca or Mg had very little effect; but substitution of the univalent bases K, $NH_4$, and Na lowered the heat of wetting appreciably, Na being somewhat less effective than K or $NH_4$. Further data of a similar nature are given in a subsequent paper by Parker and Pate (*24*).

The effect of base substitution on the electrical charge has been reported by Mattson (*18*, *19*). Sodium markedly increased the negative charge of an isolated colloidal soil material. Ca reduced the charge somewhat, and methylene blue, when added to the point of complete absorption, rendered the colloid practically isoelectric.

The viscosity and stability of a colloidal soil material saturated with different bases has been studied by Gallay (*7*), by Wiegner, Gallay, and Gessner (*31*), and by Wiegner (*30*). Both the viscosity and resistance to flocculation of colloidal soil suspensions showed a small but evident variation with the kind of substituted cation, these properties increasing in the order $Ca<K<NH_4<Na<Li$. Wiegner (*30*) correlated variations in these properties with variations in the hydration values of the ions.

There is an extensive literature dealing with the effects of various salts, acids, and alkalies on the permeability of soils and on the flocculation or electrical charge of soil particles. These studies are not reviewed, since they are only indirectly related to the present investigation. The effects observed when a salt is added to a soil and the added salt and products of reaction are not subsequently removed by leaching, are evidently partly due to an incomplete substitution of bases and partly due to the presence of the added salt.

It seems to be established by previous work that substitution of monovalent bases for the exchangeable bases present in the colloidal soil material increases the swelling, dispersion, electrical charge, viscosity, and resistance to flocculation of the material, but decreases the heat of wetting. The influence of the various actions on swelling, viscosity, etc., seems to increase in the order $Ca<K<NH_4<Na<Li$, while there is some evidence of a reverse order for the influence on heat of wetting. Little or no change seems to be produced by the substitution of Ca or Mg. Practically no data are available regarding the comparative magnitudes of the effects produced in different kinds of colloidal soil materials; since in some studies the determinations were made on the whole soil, the kind of colloid present being unknown, and in other studies only one or two different kinds of colloidal soil material were used.

## MATERIALS AND METHODS

The seven colloidal soil materials used in this investigation were selected from approximately 100 that have been studied in prior investigations in this bureau. Although they do not represent all the peculiarities in composition and properties that have been encountered, they cover fairly well the more important variations. The soils from which the colloids were isolated are all well-known types of the United States, except the Nipe, which is a pronounced lateritic soil of Cuba. This has been described by Bennett and Allison (*3*). Descriptions of the United States samples are given in previous publications (*1*, *27*).

The chemical compositions of the seven colloids are shown in Table 1. The data on ultimate composition were taken from previous publications (*1*, *27*) except in the case of the Nipe colloid. Thanks are due to G. Edgington for this analysis. The contents of exchangeable monovalent and divalent bases of the different colloids are also given in Table 1. These were determined by treating the material with normal ammonium chloride essentially according to the method described by Kelley and Brown (*16*) for soils. Exchangeable hydrogen, if present, would not be determined by this procedure. Prior to the determination the untreated colloids were leached with water for the same length of time as the materials saturated with various bases.

It will be seen that the different colloids cover a wide range in chemical composition, extreme and average values of the $\frac{Si\,O_2}{Al_2\,O_3+Fe_2\,O_3}$ ratio and of exchangeable base content being represented.

TABLE 1.—*Chemical composition and exchangeable monovalent and divalent bases of colloidal soil materials*

| Source of colloid | Ultimate chemical composition expressed in per cent | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $SiO_2$ | $TiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | MnO | CaO | MgO | $K_2O$ | $Na_2O$ | $P_2O_5$ | $SO_3$ |
| Fallon loam soil | 50.49 | 0.51 | 16.73 | 10.77 | 0.121 | 2.36 | 5.35 | 2.24 | 0.54 | 0.37 | 0.11 |
| Sharkey clay soil | 50.13 | .46 | 21.70 | 8.70 | .035 | 1.48 | 2.54 | 1.88 | .24 | .69 | ..... |
| Marshall silt loam soil | 44.94 | .47 | 22.15 | 8.91 | .126 | 1.12 | 1.95 | 2.07 | .19 | .70 | .21 |
| Sassafras silt loam subsoil | 40.93 | .70 | 29.55 | 11.98 | .033 | .37 | 1.37 | 1.04 | .31 | .11 | .02 |
| Norfolk fine sandy loam subsoil | 38.46 | .93 | 33.38 | 11.43 | .005 | .28 | .46 | .55 | .31 | .28 | .05 |
| Cecil loam subsoil | 31.84 | .53 | 38.28 | 10.04 | .136 | .36 | .23 | .46 | .28 | .11 | .02 |
| Nipe clay soil | 10.19 | ..... | 15.84 | 62.51 | ..... | .23 | .05 | trace. | trace. | ..... | ..... |

| Source of colloid | Ultimate chemical composition expressed in per cent | | | Mols $SiO_2$ / Mols $Al_2O_3+Fe_2O_3$ (ratio) | Exchangeable monovalent and divalent bases expressed as milliequivalents per gram of colloid | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ignition loss | Organic matter | $H_2O$ at 110° C. | | Ca | Mg | K | Na | Total | Total exchange capacity determined with $CaCl_2$ |
| Fallon loam soil | 10.05 | 1.79 | 12.15 | 3.62 | 0.768 | 0.209 | 0.050 | 0.035 | 1.062 | 1.126 |
| Sharkey clay soil | 12.56 | 3.83 | 11.46 | 3.11 | .464 | .179 | .032 | .006 | .681 | .771 |
| Marshall silt loam soil | 16.86 | 7.94 | 8.96 | 2.73 | .330 | .144 | .039 | .026 | .539 | .605 |
| Sassafras silt loam subsoil | 14.44 | 1.88 | 5.07 | 1.85 | .157 | .056 | .004 | .010 | .277 | .243 |
| Norfolk fine sandy loam subsoil | 13.16 | 2.18 | 3.51 | 1.60 | .046 | .025 | trace. | trace. | .071 | .148 |
| Cecil loam subsoil | 18.00 | 1.44 | 4.93 | 1.20 | .060 | .035 | do | do | .095 | .098 |
| Nipe clay soil | 9.86 | ..... | ..... | .31 | .025 | trace. | do | do | .025 | .039 |

These colloidal materials were isolated from the soils essentially as described in a previous publication (*12, p. 16*), except that only a small part of the total colloid was removed. The colloid was dispersed by gently rubbing the soil and treating with distilled water to which sufficient ammonia had been added to bring the extract to a pH of approximately 7.0. The dispersed material was passed through a supercentrifuge at a sufficiently slow rate to yield a submicroscopic product; it was collected on the outside of a Pasteur-Chamberland filter, and dried on a steam bath with a very slow flow of steam.

The substitution of different cations for the exchangeable bases present in the colloids was brought about by treating 25 to 50 grams of the material with a twentieth normal solution of HCl, a 0.6 per cent solution of methylene blue (tetramethyl thionine chloride) or with neutral normal solutions of $CaCl_2$, $MgCl_2$, KCl, or NaCl. Each lot of colloid was treated twenty times with one of the above solutions, the solution being drawn off through a Pasteur-Chamberland filter after each addition. The samples were then washed with distilled water in the same manner until the wash water gave no test for chloride. The time required for substitution of cations and removal of the excess solution varied from a few days in the case of the Nipe and Cecil colloids to as long as four to six weeks in the case of the Fallon, Sharkey, and Marshall colloids treated with NaCl. The untreated samples of the different colloids were washed for the same length of time as the calcium-saturated samples. The pH values of the filtrates were determined colorimetrically from time to time as the washing proceeded. The sodium-chloride-treated samples of the Sharkey and Marshall colloids gave neutral wash waters at first, but as washing proceeded the pH rose to 7.6 and the sodium-chloride-treated Fallon increased to a pH of about 9.5. The alkaline filtrates were somewhat dark with organic matter. This is in accord with the experience of Cummins and Kelley (*6*) and others. Filtrates of the other NaCl treated colloids showed little change in pH during the washing. The distilled water used had a pH of about 5.8.

The colloidal materials prepared in this way were examined for heat of wetting, capacity for adsorbing water vapor, swelling, moisture equivalent, cataphoretic velocity, and pH. It was presumed that the influence of exchangeable cations on properties would be especially apparent in these determinations, since they had disclosed well-defined variations in different untreated colloidal soil materials. The procedures followed in making the various determinations were essentially the same as those described in detail in an earlier publication (*1*), hence only brief comment is required here. This is given below.

In determining the heat of wetting of certain colloids saturated with sodium it was necessary to allow 10 minutes for the temperature to attain a maximum, instead of the usual 5 minutes, owing to slow penetration of the water.

The adsorption of water vapor was determined over 30 per cent (by weight) sulphuric acid rather than over 3.3 per cent acid, since previous work showed that different soil colloids may adsorb widely different quantities of water from a comparatively dry atmosphere, although they adsorb approximately equal quantities from a nearly saturated atmosphere.

In determining swelling, or volume increase in water, the apparent volume which the coarsely pulverized, dried colloid occupies in water is compared with the apparent volume in benzene. The method measures roughly the increase in total volume of pore space brought about by the action of water. Some idea of the mechanism of this change can be obtained by observing the material under a low-power microscope. When dried aggregates of colloidal soil material are wetted there is more or less shattering into smaller fragments, and ni the case of certain colloids there is some obvious swelling of these fragments without obvious shattering. The swelling of the fragments without visible shattering may be due to a uniform increase in the sizes of the finer microscopic and submicroscopic pores already present in the fragments or it may be due to a further, irregular shattering into aggregates so small that they are not distinguishable with a low-power microscope. Thus in the course of swelling the grosser voids formed by juxtaposition of particles of the dried powder may be replaced by a number of smaller voids owing to a breaking up of aggregates and the finer pores may be considerably enlarged. Since considerable shattering into aggregates distinguishable under the lower-power microscope takes place in colloidal materials that show little increase in apparent volume, it is obvious that the marked increases in apparent volume that occur in certain colloids are due chiefly to enlargement of the finer capillaries or to submicroscopic shattering. This method for swelling, which is only an approximation, and the more accurate moisture-equivalent determination indicate somewhat similar characteristics of the colloidal material. The swelling method shows approximately the increase in the total pore space brought about by saturation with water, while the moisture-equivalent method indicates that pore space of the material after swelling which will hold water against 1,000 gravity. These determinations in the case of soil colloids may be regarded as indicating roughly the tendency of the material to become more disperse on the mere addition of water. In previous work a marked parallelism was found between these two determinations in the case of seven different colloids.

The apparatus used for measuring the cataphoretic movement or migration velocity of the colloids was like that described in earlier work except that the capillary tube had a diameter of 3 millimeters instead of 2 millimeters. The movement of the particles was observed in the annular layer 0.42 of the radius from the wall of the tube. Since on observing velocities of particles at different depths in the tube it was found that at this point the velocity was the mean of those occurring at different depths from the wall to the axis of the tube. The absolute values obtained in such a tube should not be regarded as necessarily possessing a high degree of accuracy, but the results are comparable.

For the hydrogen-ion determination, a hydrogen electrode was used, and the proportion of air-dried colloid to water was 1 to 3, except in the case of certain sodium-saturated colloids in which swelling was so great that a proportion of 1:5 was necessary.

## EXPERIMENTAL RESULTS

The properties of the different colloidal materials, determined by the methods previously described, are shown in Table 2. Values given in this table were reproducible within 1 to 5 in the last decimal places reported when the same lot of colloid was used, except in the case of high values for swelling and moisture equivalent; nevertheless, small differences in properties should probably be considered characteristic effects of the cations only when they occur fairly consistently in the colloids of different soils. Properties of colloids saturated with cations by neutral salt treatment vary somewhat with the thoroughness of subsequent washing to remove excess salt, and some differences in properties may be due to possible inequalities in washing rather than to a specific cation effect. An attempt was made, however, to bring the differently prepared colloids to a comparable condition by washing until the wash water gave no test for chlorides.

TABLE 2.—*Properties of soil colloids when saturated with different bases*

| Kind of colloid | Substituted cation | Heat of wetting per gram of colloid | $H_2O$ vapor adsorbed over 30 per cent sulphuric acid per gram of colloid | Swelling | Moisture equivalent | Migration velocity of colloid particles in distilled water | pH |
|---|---|---|---|---|---|---|---|
| | | *Calories* | *Gram* | *Per cent* | *Per cent* | *μ second* | |
| Fallon | None | 18.8 | 0.171 | 120 | 100 | −2.3 | 8.4 |
| | Ca | 18.4 | .174 | 130 | 95 | −2.1 | 8.4 |
| | Mg | 17.8 | .177 | 110 | 103 | −2.1 | 8.4 |
| | K | 10.1 | .132 | 110 | 102 | −3.8 | 9.1 |
| | Na | 13.4 | .171 | 210 | 228 | −4.3 | 9.6 |
| | H | 15.2 | .173 | 60 | 78 | −Trace. | 4.0 |
| | Methylene blue | 6.7 | .077 | 9 | 64 | −Trace. | 6.2 |
| Sharkey | None | 17.4 | .150 | 80 | 85 | −2.2 | 6.1 |
| | Ca | 17.8 | .152 | 70 | 81 | −2.2 | 7.1 |
| | Mg | 20.0 | .150 | 70 | 96 | −2.2 | 6.3 |
| | K | 10.3 | .115 | 95 | 90 | −3.2 | 6.8 |
| | Na | 13.4 | .146 | 230 | 201 | −4.5 | 6.8 |
| | H | 13.7 | .153 | 60 | 78 | −1.7 | 3.5 |
| | Methylene blue | 5.1 | .074 | 14 | 66 | −1.0 | 4.0 |
| Marshall | None | 13.6 | .134 | 67 | 77 | −2.4 | 6.3 |
| | Ca | 14.4 | .136 | 67 | 79 | −2.5 | 6.3 |
| | Mg | 14.0 | .139 | 62 | 81 | −2.4 | 6.6 |
| | K | 9.9 | .115 | 71 | 103 | −4.7 | 6.3 |
| | Na | 12.3 | .141 | 124 | 212 | −4.8 | 7.6 |
| | None | 10.4 | .101 | 64 | 67 | −2.4 | 6.4 |
| Sassafras | Ca | 10.3 | .104 | 55 | 67 | −2.3 | 6.5 |
| | Mg | 10.2 | .100 | 59 | 66 | −2.2 | 6.5 |
| | K | 8.2 | .086 | 64 | 67 | −2.4 | 6.6 |
| | Na | 8.9 | .097 | 77 | 84 | −2.7 | 7.2 |
| | None | 7.8 | .081 | 40 | 67 | −2.0 | 5.9 |
| Norfolk | Ca | 8.1 | .087 | 44 | 67 | −1.9 | 6.0 |
| | Mg | 8.3 | .086 | 44 | 67 | −1.9 | 5.5 |
| | K | 7.0 | .077 | 48 | 70 | −1.9 | 6.0 |
| | Na | 7.7 | .083 | 56 | 82 | −2.0 | 6.8 |
| | H | 7.3 | .085 | 36 | 71 | Isoelectric | 4.1 |
| | Methylene blue | 4.7 | .027 | Trace. | 78 | +.8 | 4.2 |
| Cecil | None | 5.1 | .042 | 28 | 56 | −2.3 | 6.5 |
| | Ca | 5.7 | .052 | 28 | 58 | −2.2 | 6.7 |
| | Mg | 5.2 | .050 | 28 | 57 | −2.3 | 6.7 |
| | K | 4.8 | .041 | 28 | 58 | −2.6 | 6.9 |
| | Na | 5.0 | .045 | 28 | 57 | −2.4 | 7.0 |
| | H | 4.1 | .049 | 17 | 55 | −.8 | 4.4 |
| | Methylene blue | 3.4 | .030 | Trace. | 60 | +1.2 | 4.6 |
| Nipe | None | 6.4 | .054 | Trace. | 58 | −Trace. | 6.7 |
| | Ca | 6.6 | .054 | Trace. | 56 | −Trace. | 6.9 |
| | Mg | 6.6 | .053 | Trace. | 56 | −Trace. | 6.9 |
| | K | 6.6 | .053 | Trace. | 57 | −Trace. | 6.6 |
| | Na | 6.4 | .052 | Trace. | 56 | −Trace. | 6.2 |
| | H | 5.9 | .052 | Trace. | 53 | Isoelectric | 5.4 |

It is quite evident from the data that some cations alter properties much more than others; that some properties are markedly altered while others are little affected by the different cations; and that there is a wide difference in the alterability of different soil colloids. These three generalizations will be discussed in detail under separate headings.

### THE COMPARATIVE EFFECTS OF DIFFERENT CATIONS

The comparative effects of different cations on properties can be more readily seen if the data given in Table 2 are expressed on a relative basis. In Table 3 the value for any property yielded by a calcium-saturated colloid is given as 100, and values yielded by the colloid saturated with other cations are expressed relative to 100. The relative figures for acidity do not, of course, represent comparative hydrogen-ion concentrations but comparative pH values.

TABLE 3.—*Properties of soil colloids saturated with different bases, calculated as percentage of Ca saturated values*

| Kind of colloid | Substituted cation | Heat of wetting per gram of colloid | $H_2O$ vapor adsorbed over 30 per cent sulphuric acid per gram of colloid | Swelling | Moisture equivalent | Migration velocity of colloid particles in distilled water | pH |
|---|---|---|---|---|---|---|---|
| | None | 102 | 98 | 92 | 105 | 110 | 100 |
| | Ca | 100 | 100 | 100 | 100 | 100 | 100 |
| | Mg | 97 | 102 | 85 | 108 | 100 | 100 |
| Fallon | K | 55 | 76 | 85 | 107 | 181 | 108 |
| | Na | 73 | 98 | 162 | 240 | 205 | 114 |
| | H | 83 | 99 | 46 | 82 | | 48 |
| | Methylene blue | 36 | 44 | 7 | 67 | | 74 |
| | None | 98 | 99 | 114 | 105 | 100 | 85 |
| | Ca | 100 | 100 | 100 | 100 | 100 | 100 |
| | Mg | 112 | 99 | 100 | 119 | 100 | 89 |
| Sharkey | K | 58 | 75 | 146 | 111 | 145 | 96 |
| | Na | 75 | 96 | 329 | 252 | 205 | 96 |
| | H | 77 | 101 | 86 | 98 | 77 | 49 |
| | Methylene blue | 29 | 49 | 20 | 81 | 45 | 56 |
| | None | 94 | 99 | 100 | 97 | 96 | 100 |
| | Ca | 100 | 100 | 100 | 100 | 100 | 100 |
| Marshall | Mg | 97 | 103 | 93 | 103 | 96 | 105 |
| | K | 69 | 85 | 106 | 130 | 188 | 100 |
| | Na | 85 | 104 | 185 | 268 | 192 | 121 |
| | None | 101 | 97 | 116 | 100 | 104 | 98 |
| | Ca | 100 | 100 | 100 | 100 | 100 | 100 |
| Sassafras | Mg | 99 | 97 | 107 | 99 | 96 | 100 |
| | K | 80 | 83 | 116 | 100 | 104 | 102 |
| | Na | 86 | 94 | 140 | 125 | 118 | 111 |
| | None | 96 | 93 | 91 | 100 | 105 | 98 |
| | Ca | 100 | 100 | 100 | 100 | 100 | 100 |
| | Mg | 102 | 99 | 100 | 100 | 100 | 92 |
| Norfolk | K | 86 | 89 | 109 | 104 | 100 | 100 |
| | Na | 95 | 96 | 127 | 122 | 105 | 113 |
| | H | 90 | 98 | 82 | 106 | 0 | 68 |
| | Methylene blue | 58 | 32 | 0 | 116 | | 70 |
| | None | 89 | 82 | 100 | 97 | 104 | 97 |
| | Ca | 100 | 100 | 100 | 100 | 100 | 100 |
| | Mg | 91 | 96 | 100 | 98 | 104 | 100 |
| Cecil | K | 84 | 80 | 100 | 100 | 118 | 103 |
| | Na | 88 | 88 | 100 | 98 | 109 | 104 |
| | H | 72 | 94 | 81 | 95 | 36 | 66 |
| | Methylene blue | 60 | 59 | 0 | 103 | | 69 |
| | None | 97 | 99 | 100 | 104 | | 97 |
| | Ca | 100 | 100 | 100 | 100 | | 100 |
| Nipe | Mg | 100 | 99 | 100 | 100 | | 100 |
| | K | 100 | 98 | 100 | 102 | | 96 |
| | Na | 97 | 96 | 100 | 100 | | 90 |
| | H | 89 | 96 | 100 | 95 | | 78 |

Several characteristic effects of the various cations on properties are immediately apparent. Magnesium differs only slightly from calcium in its influence on the six properties studied. The methylene blue cation is unique in reducing practically all properties most strongly. The hydrogen ion has an influence approaching that of methylene blue, except in the cases of heat of wetting and moisture adsorption. Sodium is the only cation of those studied that gives markedly higher values than the Ca ion, but it does not have this effect on heat of wetting or moisture adsorption. The potassium ion is the most variable in its effects; it is next to methylene blue in reducing heat of wetting and moisture adsorption; it approaches sodium in increasing migration velocity, and it differs little from Ca and Mg in its influence on other properties. These generalizations, of course, apply to the results of Table 3 and may not hold for other properties of the colloidal soil material.

The order in which the different ions affect each of the six properties is best shown by averaging the results yielded by the various soils. This summary of the comparative effects of the cations is given in Table 4.

TABLE 4.—*The comparative effects of different cations on six properties of soil colloids saturated with different bases*

| Property | Relative values yielded by colloids saturated with— | | | | | |
|---|---|---|---|---|---|---|
| | Ca | Mg | K | Na | H | Methylene blue |
| Water adsorbed | 100 | 99 | 84 | 96 | 98 | 46 |
| Heat of wetting | 100 | 100 | 76 | 86 | 82 | 46 |
| Swelling | 100 | 98 | 109 | 163 | 75 | 7 |
| Moisture equivalent | 100 | 104 | 108 | 172 | 95 | 92 |
| Migration velocity | 100 | 99 | 134 | 149 | 23 | −13 |
| pH | 100 | 98 | 101 | 107 | 62 | 67 |

The same order of cations seems to apply to water adsorption as to heat of wetting, although water adsorption shows on the whole less pronounced variations than the heat of wetting. This order is approximately Ca, Mg, Na, H, K, methylene blue, the last ion giving the lowest values. It is apparent from Table 3 that differences between Ca and Mg and between Na and H are small in the case of each soil colloid—about what should be allowed for experimental error. But except for occasional transpositions in these pairs of ions, the above order holds consistently for those soil colloids showing alteration. This order is in agreement with the order, Ca>Mg>Na>K, found by Pate (*25*) for ion effects on heat of wetting.

A quite different order holds for effects of the cations on swelling, moisture equivalent, migration velocity, and pH; namely Na, K, Ca, Mg, H, methylene blue, arranged in descending order. Here again differences between Ca and Mg are slight, and K is close to Ca and Mg in its effect on swelling and pH, but the series is well defined in other respects and undoubtedly significant. This series corresponds to the order Na>$NH_4$>K>Ca observed by Wiegner (*30*) in studies of viscosity and stability of suspension, and it agrees with the order Na<K<$NH_4$<Ca given by Gedroiz (*11*) for the energies with which neutral salts replace exchangeable cations.

Such orders of ion effects are usually ascribed to differences produced in the structure of the colloidal particles or micelles, especially to changes in the Helmholtz double layer. An explanation for such changes is furnished by the current theory of ion effects in colloidal systems as presented by Michaelis (*22*). The colloidal soil material, washed free from excess soluble salts, evidently falls in the category of those comparatively insoluble substances which have a tendency to dissociate into ions. Such substances, according to theory, dissociate at the surface large, insoluble ions, which remain in the surface, and smaller, soluble ions of opposite charge, which are in true solution, but are held in a layer near the insoluble surface ions by electrostatic attraction. The density and diffuseness of the outer layer of ions depends on the tendency of the colloid to ionize. In the case of the colloidal soil material, the chief large ion remaining in the surface of the particle is evidently some form of silicate anion and part, at least, of the substituted cations constitute the outer layer of ions. The colloids with the different substituted cations may be supposed to have a decreasing tendency to ionize in the order Na, K, Ca, Mg, H, methylene, blue and consequently a decreasing density and diffuseness of the double layer.

The significant feature of this concept concerning the conditions of the colloid when saturated with different cations is the variation in ionization tendency produced by different substituted cations. This feature is quite generally accepted even by those who do not go further in discussing a double layer. Gedroiz (*8*), for instance, speaks of the sodium saturated colloid as having a greater "elasticity of solubility" than the calcium saturated material. Kelley and Brown (*17*) regard the Na complex as being more soluble and of a greater tendency to hydrolysis and ionization than the calcium combination, and Bradfield (*5*) regards the combination of the colloid with NaOH as more soluble than that produced by neutralization with $Ca(OH)_2$. In 1922 Hissink (*13*) described the soil colloidal micelle in terms very similar to those used above and suggested that the Ca and Mg combinations are insoluble and little ionized as compared with the Na and K combinations. Wiegner (*30*) has gone further in attributing differences in the diffuseness of the double layer to differences in the hydrations of the ions. Monovalent ions, such as K and $NH_4$, that are little hydrated are held closer to the surface of the particle and give less diffuse double layers than more highly hydrated ions, such as Na and Li.

Previous work dealing with the effects of different substituted cations on the isolated colloidal soil material has been conducted with materials in which the substitution has been practically complete. Thus, most of the direct measurements of cation effects have been made on materials containing only one kind of exchangeable cation. It is quite important to observe also the extent to which properties of the isolated colloidal material are affected by a partial substitution of cations; since this should throw light on the results that may be expected to follow applications of salts in actual field practice. The general idea at present seems to be that each cation exerts its characteristic influence in proportion to the quantity present. At least, certain ratios of exchangeable cations in the whole soil are supposed to indicate the properties of the colloid present, and such ratios could hardly be significant unless the influence of the

cation is proportional to its quantity. This seems a reasonable assumption, but it should be verified.

In order to gain some idea of the effect of a partial substitution of cations, Fallon and Sharkey colloids previously saturated with a single cation were again treated with a neutral salt to bring about a partial replacement. The excess of the added salt was removed by washing until the wash water was free of chlorides, as in the case of the first substitution. Twentieth normal sodium chloride was used to partially substitute Na for Ca or Mg in all cases except one. In this case $CaCl_2$ was used to partially replace Na in the Na saturated colloid. Measurements were made of the migration velocity, swelling, and moisture equivalent of the new preparations, since these properties are markedly affected by complete substitution. The values obtained are given in Table 5. In order to show how closely the observed values agree with the idea that the influence of a cation is proportional to the quantity present, values calculated on this assumption are also given in the table.

TABLE 5.—*Variation in properties of soil colloids saturated with different bases by partial substitution of cations*

[Observed values and values calculated on the assumption that properties vary directly with the proportion of the base substituted]

| Kind of colloid | Exchangeable bases present | Migration velocity value | | Moisture equivalent value | | Swelling value | |
|---|---|---|---|---|---|---|---|
| | | Observed | Calculated | Observed | Calculated | Observed | Calculated |
| | | *μ seconds* | *μ seconds* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Fallon | Na saturated | 4.3 | | 228 | | 210 | |
| Do | 31 per cent Na, 69 per cent Ca. | 2.6 | 2.8 | 131 | 136 | 150 | 155 |
| Do | 19 per cent Na, 81 per cent Ca.* | 2.0 | 2.5 | 100 | 120 | 140 | 145 |
| Do | 13 per cent Na, 87 per cent Ca. | 2.4 | 2.4 | 103 | 112 | 140 | 140 |
| Do | Ca saturated | 2.1 | | 95 | | 130 | |
| Do | 29 per cent Na, 71 per cent Mg. | 3.1 | 2.7 | 237 | 139 | 160 | 139 |
| Do | Mg saturated | 2.1 | | 103 | | 110 | |
| Sharkey | Na saturated | 4.5 | | 201 | | 230 | |
| Do | 28 per cent Na, 72 per cent Ca. | 2.9 | 2.8 | 213 | 125 | 140 | 115 |
| Do | Ca saturated | 2.2 | | 95 | | 70 | |

* Calcium substituted in sodium-saturated colloid.

Except for two moisture-equivalent determinations, the values obtained for properties indicate that a property is altered by a substituted cation in direct proportion to the degree of replacement. Values calculated on this basis in most cases agree as closely as could be expected with values actually obtained, considering the accuracy of the determinations. However, the moisture equivalents of the Fallon 29 per cent Na, 71 per cent Mg, and of the Sharkey 28 per cent Na, 72 per cent Ca are practically the same as the values yielded by the completely saturated materials, indicating that the partial substitution of a cation may have as pronounced an effect on a property as a complete saturation. Sufficient material was not available to repeat these two determinations, but there is no reason for regarding them as erroneous, except that they are not in harmony

with the 13 other determinations. Further data on this subject are obviously desirable, but the present data indicate that the influence of a substituted cation on properties is usually proportional to the extent of its substitution.

### ALTERATION IN DIFFERENT PROPERTIES

The various properties that have been determined might be considered as falling into two groups, heat of wetting and moisture adsorption forming one group and the remaining properties a second. It will be recalled that quite different orders of cation effects obtained for these two groups. The heat of wetting and moisture adsorption tended to be reduced by the substitution of Na or K; whereas the other properties were, as a rule, markedly increased by these cations. Also, the first two properties are primarily those of the comparatively dry, undispersed material, whereas swelling, moisture equivalent, migration velocity, and pH are properties of the material in a state of complete or incipient dispersion. It is true that an excess of water is present in the heat-of-wetting determination; but it is evident from the results of Mitscherlich (*23*) and of Bouyoucos (*4*) that practically all the heat evolved is associated with the adsorption of a small quantity of water; hence it may be regarded as a property of the nearly dry, aggregated material.

The lowering of heat of wetting and of moisture adsorption produced by substitution of K, Na, and methylene blue is not readily explained. As pointed out in a previous publication (*1*), the adsorption of water vapor and evolution of heat on wetting by materials similar to dry soil colloids are supposed to be dependent on the size and the total volume of submicroscopic capillaries present and on the kind of surface exposed in the capillaries. It seems probable that the adsorption of methylene blue might clog the finest capillaries or present a surface of diminished affinity for water. However, K and Na would not be expected to have similar effects. The adsorption of these ions would be presumed rather to increase the affinity of the surface for water, since they are regarded as highly hydrated ions that give comparatively soluble combinations with the colloid. Neither would K and Na be expected to reduce the total volume of fine capillaries, since these ions tend to increase the dispersion of the colloid. It should be borne in mind, however, that the structure of the material presumably concerned in these determinations is that produced by drying down of dispersed material, and it is not inconceivable that a highly dispersed colloid of greater solubility should, under this treatment, give a material with a smaller volume of fine pores than material originally more aggregated and insoluble.

There is some evidence that the lowered heat of wetting of the K and Na colloids is due to a reduction in the surface or volume of capillaries, but it is far from conclusive. When the heat of wetting of several of the colloids in absolute alcohol and benzene were determined, it was found that the K colloids gave appreciably lower values than the Ca colloids in these liquids also. This rather indicates a reduction in pore space; but the results could also be attributed to a change in the surface attraction for benzene and alcohol.

Pate (*25*), who observed a similar lowering of heat of wetting on substitution of potassium, suggested that it might be due in part to a loss of organic matter brought about in washing the material free

from soluble salts. In the substitution of different cations and the subsequent washing slight losses of silica do occur and some organic matter also is lost, as is shown by the dark color of the final washings. It was found, however, that these losses could account for only a small portion of the reduction in heat of wetting attendant on the substitution of potassium. The three colloids, heats of wetting of which were reduced most by substitution of potassium, the Fallon, Sharkey, and Marshall colloids, were treated with $CaCl_2$ to replace with Ca the K previously introduced. The heats of wetting of these three new Fallon, Sharkey, and Marshall colloids were, respectively, 84, 90, and 100 per cent of the values given by the Ca preparations that had never undergone K substitutions. Furthermore, the black Marshall colloid, which seemed to lose most organic matter, was the colloid which showed no reduction in heat of wetting when put through this cycle of substitution.

Variations in swelling, moisture equivalent, and migration velocity are all readily explained by the current theory regarding the solubility of the differently substituted materials and attendant differences in the double layer surrounding the particles. The migration velocity, which is supposed to vary directly with the electrical charge of the particle, is an indirect measure of differences in the density and diffusivity of the double layer formed by the substituted cations. And it is apparent from the data given in Tables 2 and 3 that most colloids with different substituted cations show wide variations in this property.

Variations in the swelling and moisture-equivalent values (Tables 3 and 4) produced by the different cations are quite similar, except in the case of methylene blue. This cation reduced swelling much more markedly than it did the moisture equivalent. These results are readily understood if it is borne in mind that the moisture-equivalent determination is an expression of the total pore space (capable of holding water against 1,000 gravity) in the material after it has swelled, while the swelling determination measures only an increase in pore space produced by dispersion or swelling of the material in water. Table 2 shows that most of the Ca colloids swell appreciably in water and that the methylene blue colloids have practically no tendency to disperse or swell. The "unswelled" pore space of the two colloids, which is included in the moisture-equivalent determination, is probably about the same and is obviously greater than the "swelling" space of most Ca colloids; hence methylene blue does not greatly reduce the moisture equivalent, although it practically eliminates swelling.

If swelling is regarded as the tendency of a material to become more dispersed, it is obvious that a treatment, such as a substitution of cations, which increases the charge on the particles may be expected to increase swelling. In the case of proteins, which swell markedly as compared with soil colloids, a close connection between electrical charge and swelling has been shown by Loeb. Possibly a somewhat closer correspondence between swelling and migration velocity would have been found in this work if a more exact method for determining the former had been available, although a very close correspondence is not to be looked for in materials that are as slightly reversible as soil colloids.

Variations in the acidity of soil colloids saturated with different cations are roughly explained by the current idea that the exchange-

able cations are wholly or partly combined in some way with complex silica, silicate, or organic anions. Whether the anion is of definite composition and whether the combination with the cation is the same as that obtaining in ordinary definite chemical compounds or only similar are not essential in this connection. Also the relation of these ions to the colloidal micelle need not be considered in explaining the pH values in a qualitative manner. The hydrogen-saturated colloid may be regarded as acid, owing to a slight solubility and ionization of the H-anion combination; while the pH values of the Ca, Mg, K, and Na colloids may be considered as depending on the completeness with which H is substituted, on the tendency of the combination to ionize, and on the extent of the hydrolysis of the constitutents.

The data of Table 2 show that the H and methylene blue colloids are markedly acid in reaction, that the Ca and Mg colloids vary from weakly acid to slightly alkaline, and that the K and Na colloids are as a rule slightly more alkaline than the Ca and Mg materials. The acidity of the methylene blue material is doubtless due to the fact that the tetramethylthionine chloride used to saturate the colloid was slightly acid; hence there was a substitution of H ions as well as of methylene blue cations. The higher pH of the K and Na colloids than of the Ca and Mg colloids may be ascribed to an increased solubility and hydrolysis of the monovalent cation combinations. Although the theory of the double layer is not needed to explain the pH results in a general way, it is quite evident that such a layer must exist; and this being the case, the concentration of H or Ca ions near the surface of the particle would be greater than in the bulk of the solution. This idea is in harmony with the results of Rice and Osugi (*26*), who found that a greater inversion of cane sugar took place in contact with the solid particles of a suspension of neutral or acid soils than with soil extracts alone.

Many of the pH values given in Table 2 seem anomalous. In the case of the Sharkey and Nipe colloids, the pH of the Na material is less than that of the Ca preparation; some of the Ca colloids are slightly acid rather than alkaline; and different colloids saturated with the same cation show wide differences in pH in some instances. Probably little significance should be attached to these variations in view of the manner in which the colloids were prepared. Washing the differently treated colloids to remove excess salt was a long drawn-out procedure and the ordinary distilled water used in the process usually had a pH of approximately 5.8. Doubtless before the excess salt was completely removed by leaching, some of the cations in combination with the colloid as well as those in combination with Cl were removed through a substitution of H ions in the water. The appearance of alkalinity in the wash water is, of course, evidence of this. A sufficiently prolonged leaching would obviously have brought all materials to the same pH as that of the water, about 5.8. The Na and K colloids would be expected to have their pH values affected more by washing than the Ca materials; also a certain amount of washing would probably affect colloids of a low-exchange capacity differently from colloids containing more exchangeable bases. Probably in view of the somewhat crude control exercised over the washing, only the general trend of the results that has been discussed should be considered significant and not the lesser variations.

ALTERATION IN DIFFERENT SOIL COLLOIDS

A marked feature of the data given in Table 2 is the difference in alterability of the seven soil colloids. In the case of the Fallon and Sharkey colloids a substitution of certain cations obviously alters properties considerably; whereas in the case of the Cecil and Nipe colloids properties are comparatively unaffected by the substitution of any cation. A closer comparison of the various colloids for differences in range or capacity of alteration can be made on the basis of data given in Table 6. This table shows for each soil colloid differences between the Ca and K preparations in heat of wetting and in moisture adsorption and differences between Na and Ca preparations in swelling, moisture equivalent, and migration velocity. These differences, indicative of capacity for alteration, are also shown calculated as percentages of the values yielded by the calcium preparations.

TABLE 6.—*Ranges of alteration of the soil colloids as shown by differences in properties of the K, Ca, and Na preparations*

| Kind of colloid | Heat of wetting of Ca preparation minus value for K preparation | $H_2O$ adsorbed by Ca preparation minus value for K preparation | Swelling of Na preparation minus value for Ca preparation | Moisture equivalent of Na preparation minus value for Ca preparation | Migration velocity of Na preparation minus value for Ca preparation | Differences expressed as percentage of value shown by Ca preparation in— | | | | | Exchangeable base content per gram of Ca colloid | Molecular ratio of $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Heat of wetting | $H_2O$ adsorbed | Swelling | Moisture equivalent | Migration velocity | | |
| | *Calories* | *Gram* | *Per cent* | *Per cent* | *μ/seconds* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Milli-equivalent* | |
| Fallon | 8.3 | 0.042 | 80 | 133 | 2.2 | 45 | 24 | 62 | 140 | 105 | 1.126 | 3.62 |
| Sharkey | 7.5 | .037 | 160 | 120 | 2.3 | 42 | 24 | 229 | 148 | 105 | .771 | 3.11 |
| Marshall | 4.5 | .021 | 57 | 133 | 2.3 | 31 | 15 | 85 | 168 | 92 | .605 | 2.73 |
| Sassafras | 2.1 | .018 | 22 | 17 | .4 | 20 | 17 | 40 | 25 | 18 | .243 | 1.85 |
| Norfolk | 1.1 | .010 | 12 | 15 | .1 | 16 | 11 | 27 | 22 | 5 | .146 | 1.60 |
| Cecil | .9 | .011 | 0 | 0 | .2 | 16 | 21 | 0 | 0 | 9 | .098 | 1.20 |
| Nipe | 0 | .001 | 5 | 0 | 0 | 0 | 2 | 0 | 2 | ------ | .039 | .31 |

Differences between the Ca and the K or Na preparations show that the Fallon colloid has, on the whole, the greatest latitude of alteration and that each succeeding colloid in the table has a progressively smaller range. The alteration ranges of the various colloids are more regularly graded in heat of wetting and moisture adsorption than in other properties; but all properties indicate about the same order for the different colloids. If this order for range of alteration is now compared with the order in which the various Ca or untreated colloids stand with respect to the degree they possess a given property (Table 2), it will be seen that the two orders are practically identical. Evidently the higher the heat of wetting, moisture adsorption, etc., of a colloid the greater is the alteration that can be produced by a substitution of cations.

A further generalization regarding range of alteration is indicated by the figures in Table 6 which show ranges of alteration calculated as percentages of the property values of the Ca colloid. It will be noted that the order of the colloids indicated by these figures is

usually the same as the order in which the Ca or untreated colloids stand (Table 2) with respect to magnitude of properties. It appears, then, that as properties of untreated or Ca saturated colloids increase in magnitude, the range of alteration forms an increasing proportion of this magnitude.

The range of alteration that may be obtained by substitution of different cations is evidently just as characteristic a feature of a soil colloid as its capacity for adsorption or its specific heat of wetting. Moreover, a fairly good idea of the alteration range can be obtained from comparatively simple adsorption, moisture-equivalent, etc., determinations of the untreated colloid.

In a previous publication (*1*) it was shown that the properties of different colloidal soil materials correlated fairly well with the total exchangeable bases of the material and with the molecular ratio, $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$. It is apparent from the data in Table 6 that range of alteration likewise shows a fair correlation with these features of chemical composition. Although these relations are only approximate, it seems that little alteration in properties is to be expected on substitution of different cations, if the colloid has an exchangeable base content of less than 0.15 milli-equivalents per gram or a $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio below 1.5. These figures, of course, are only tentative; since they are based on the examination of only a few soil colloids.

Some of these relations between properties, range of alteration, exchangeable base content, and $\frac{SiO_2}{Al_2O_2+Fe_2O_3}$ ratio of soil colloids have already been discussed. It was suggested in a previous publication (*1*) that the relation between properties and silica ratio was probably not due to a direct dependence of properties on the relative proportions of silica, iron, and alumina. Many properties of the colloid are, doubtless, influenced directly by the exchangeable bases, since these give rise to an electrical double layer when sufficient water is present. However, the total exchangeable base content probably is in turn directly dependent in some measure upon the silica-iron-alumina ratio, inasmuch as the exchangeable bases are supposed to be combined in some way with the silica. The fact that the total exchangeable bases of different colloids correlate better with the molecular ratio of silica to alumina and iron than with the silica content alone indicates that iron and alumina modify the silica-exchangeable-base combination, possibly in modifying the silica or silicate surface available for the combination. That properties and range of alteration in properties correlate fairly well with the total exchangeable bases might be taken as indicating that all the exchangeable cations are present in the double layer when the colloidal material is suspended. The correlation could also be explained on the ground that the quantity of exchangeable cations at the surface of the particle is a function of the total quantity present in the colloid. Calculations by Mattson (*19*) rather indicate that only a portion of the exchangeable cations are ionized at one time.

## DISCUSSION

Range of alteration is a colloid characteristic of some practical significance, since it should indicate the kinds of soil that may be expected to show changed properties after long-continued treatment with certain fertilizers. Obviously, soils containing colloids of low-alteration ranges would not be expected to show appreciable changes in physical properties even after very heavy applications of a single fertilizing salt; providing, of course, the excess of soluble salt is removed by leaching. Soils containing colloids of high-alteration ranges, on the other hand, would be expected to show appreciable changes following such treatment, if the cation of the fertilizer were Na or K, or if the fertilizer contained or developed considerable acid. These generalizations apply only to changes in the physical condition of a soil. Quite different conclusions would be drawn with respect to effects produced on the supply of available plant nutrients.

Also, in estimating the reclamation possibilities of saline soils it would seem important to consider the alteration range of the colloid as well as the mechanical composition of the soil. It has frequently been observed that certain alkali soils become so impermeable on leaching that a complete removal of alkali salts is rendered difficult or impossible. This phenomena has been discussed at length by Gedroiz (*10*) and by Kelley and Brown (*17*). It seems to be primarily due to an increased dispersion and swelling of the colloidal soil material brought about by a substitution of Na ions. The results given in Table 2 indicate that this difficulty would not necessarily be encountered in all sodium-saturated soils. Soils containing colloids like the Norfolk, Cecil, and Nipe would evidently be little affected by saturation with any cation. Soils containing colloids like the Fallon, Sharkey, and Marshall, however, would presumably be difficult to reclaim, the properties of these materials being markedly altered by Na substitution. As a matter of fact, the Fallon soil is highly impermeable even when saturated with Ca.

Comparatively little information is available on the distribution of the different kinds of colloidal material, but it may well be that colloids of low-alteration range are uncommon in regions where soil alkali develops. Analyses made by Robinson and Holmes (*27*) indicate this. They showed that there was some correlation between a high silica ratio of the colloidal soil material and a low rainfall. However, soils saturated with sea water might well contain colloids of the low-alteration range.

A direct determination of the alteration range of a soil colloid is so long a procedure as to be impracticable for routine examinations of soils. Data given in the preceding pages, however, serve to point out those features of the colloidal soil material that best indicate not only the range of alteration but other properties of the material as well. In previous publications of the bureau it was shown that the physical properties of different soil colloids correlate fairly well with variations in the $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio and with variations in the total content of exchangeable bases. Other investigators have attached considerable importance to ratios of the different kinds of exchangeable cations present. Hissink (*13*, *15*) has suggested as indicators of properties the ratio of exchangeable Ca+Mg: K+Na and the "degree of saturation;" that is, the relation between total exchange

capacity and the sum of the monovalent and divalent bases. Kelley and Brown (*16*) have tentatively suggested that the ratio of Na to Ca + Mg may be especially important. These investigators, however, drew conclusions from determinations made on whole soils. Since the colloidal contents of the soil were not definitely known, data on the exchange capacities of the colloids and on specific properties of the colloidal materials were not available.

It would appear from the data given in Table 6 that the total base exchange capacity of a colloid, or the $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio which is correlated with it, is the first and most important factor to be considered in judging the physical properties of a colloid from the chemical composition. The influence of different substituted cations on properties is seen to be more pronounced as the exchangeable base content increases and the kind of cation apparently has no appreciable influence when the exchangeable base content falls below a certain value. Thus, the base exchange capacity indicates at what point a ratio of bases becomes significant for predicting properties and also what importance should be attached to the ratio. If a number of soil colloids had base-exchange capacities as low as those of the Norfolk, Cecil, and Nipe, it would obviously be incorrect to predict widely different properties for them, no matter how widely they differed in ratio of Na: Ca + Mg. Also, if a number of colloids had high but widely different exchange capacities, the comparative properties would not be indicated solely by the ratios of different cations. But if the colloids had fairly high and approximately equal exchange capacities, a relation between properties and some ratio of cations might be expected, particularly if the cation ratio varied widely. Results on partial substitution of cations summarized in Table 5 indicate somewhat uncertainly that slight differences in ratios would not be significant of appreciable differences in properties.

The ratio of cations to consider would seem to depend somewhat on the property to be predicted, since the results brought together in Table 4 show that one cation may influence a given property considerably more than another. Heat of wetting and moisture adsorption, for instance, are particularly affected by exchangeable K, while swelling is markedly affected by Na and H. As a matter of fact, a high proportion of exchangeable K is probably very seldom found in a natural soil colloid; a high content of Na would be found only in saline soils, and appreciable quantities of Na and H would not be looked for in the same soil colloid. In the case of saline or alkali soils, doubtless, consideration should be given to the ratio of Na to total exchange capacity, and in the case of humid or leached soils, Hissink's degree of saturation, or the ratio of exchangeable H to total exchange capacity, should be considered.

Probably for the general run of soil colloids, excepting those of saline soils, as accurate an indication of properties as can be expected will be obtained simply from the total base-exchange capacity, or from one of those determinations correlated with it, such as $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio, specific absorption capacity, specific heat of wetting, etc. The various relations that have been found for different soil colloids—relations of one property to another, and relations of properties to different features of chemical composition—are all far from

perfect; hence at best only an approximate idea of properties can be expected from these determinations. Determinations of base-exchange capacity, heat of wetting, or moisture adsorption over strong acid will probably prove most valuable in soil-reconnaissance work, since these are comparatively simple determinations. Moreover,even when applied to the whole soil, they will indicate the kind of colloid if the quantity of clay or colloid in the soil is known.

## SUMMARY

This paper deals with changes in properties that different soil colloids may undergo when the exchangeable bases are substituted by different cations. The data considered include determinations of the heat of wetting, adsorption of water vapor, swelling, moisture equivalent, cataphoresis, and pH of seven widely different colloidal materials when saturated with Ca, Mg, K, Na, H, and the cation of methylene blue.

The order of the cation effects on heat of wetting and moisture adsorption is Ca, Mg, Na, H, K, and methylene blue, the last ion giving the lowest values. The decreasing order of effects on the remaining properties is approximately Na, K, Ca, Mg, H, and methylene blue. The varying effects of the different cations are ascribed to differences in solubility and dissociation tendency of the colloid-cation combinations and to consequent differences in the density and diffusivity of the Helmholtz double layer of the colloidal micelles. The influence of a cation on a property seems to be in direct proportion to its percentage of complete replacement, but the data do not indicate this with certainty.

The different soil colloidal materials vary widely in alterability. Some soil colloids are markedly altered in properties, whereas those of other soil colloids are not appreciably affected by the substitution of any cation. The ranges of alteration of different soil colloids correlate fairly well with magnitudes of the properties of the untreated or Ca saturated materials, with the total content of exchangeable bases, and with the $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio of the colloid. These correlations are explained on the ground that many properties of soil colloids are directly influenced by the total content of exchangeable bases, and the exchangeable-base content is in a measure dependent on the $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio.

It is pointed out that range of alteration of the colloidal soil material should be considered in judging what soils would show appreciable changes in properties following heavy applications of a single fertilizing salt. Range of alteration should also prove valuable in indicating those alkali soils that will become impenetrable on leaching.

Probably in the case of most soil colloids, except those of saline soils, the properties in general will be indicated as accurately as can be expected simply by the total base-exchange capacity or by one of those determinations correlated with it, such as $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio, specific absorption capacity, specific heat of wetting, etc. These determinations, except that of the silica ratio, will indicate the kind of colloid even when applied to the whole soil, if the quantity of clay or colloid is known.

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